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ASSESSING THE STATE OF *NEPHROPS NORVEGICUS* AND MANAGEMENT MEASURES IN THE CENTRAL ADRIATIC SEA

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Abstract

Nephrops norvegicus is a sedentary bottom-dwelling crustacean that represents one of the main commercial species exploited in the Adriatic Sea (Central Mediterranean). An evaluation of the status of this important resource is thus extremely important in order to manage it in a sustainable way. The evaluation of *N. norvegicus* is complicated by several issues, mainly: (i) the complex biology and behaviour of the species itself, (ii) the presence of subpopulations with different biological traits within the same stock unit. Relevant concentration of *N. norvegicus* occurs within the Pomo/Jabuka Pits area which is characterised by peculiar oceanographic and geophysical conditions. This area represented for a long time an important fishing ground shared by Italian and Croatian fleets and recently a Fishery Restricted Area (FRA) was established there. The aim of the present study is to perform for the first time an evaluation of the status of the *N. norvegicus* subpopulation inhabiting the Pomo/Jabuka Pits also accounting for the possible effects on it of the management measures. To achieve this, the principal fishery-independent and fishery-dependent dataset available for the study area were firstly analysed and then treated. Data collected by the CNR-IRBIM of Ancona through both indirect (“UWTV”) and direct (trawling) methods were refined by means of a revision of the time series and related biases, and a modelling approach accounting for environmental and fishery effects, respectively. Commercial data for both Italy and Croatia were treated in order to obtain landings and length distributions for the Pomo area only; an historical reconstruction of data starting from 1970 was carried out for both countries. The obtained information was used as input for a Bayesian length-based stock assessment model developed through the CASAL software; the flexibility of this model is recommended for *N. norvegicus* and similar species allowing to deal with sex- and fleet-based integrated assessment method. A model was thus developed for the first time accounting both the biological features of the species (e.g. seasonal emergence behaviour) and its spatio temporal distribution. The resulted constant decreasing trend of the Spawning Stock Biomass represent a warning about the status of the subpopulation. The reliability of this assessment is supported by a good fit with the input data, which were never used before. Furthermore, the huge data treatment and the challenging model structure posed a solid base for the possible extension of this approach to the whole Adriatic.

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1. Introduction

1.1 Biology, life cycle and distribution of *Nephrops norvegicus*

Nephrops norvegicus (Linnaeus, 1758) is a decapod crustacean with a slender body shape, long claws, kidney-shaped pedunculated compound eyes, and a pronounced spinose rostrum. An unsegmented carapace covers the internal organs, the abdomen is long and segmented, ending with the telson, a false somite that enables the lobster to swim; the second to fifth pereiopods constitute the walking legs, while the pleopods on the abdomen are used both for swimming and for reproductive purposes (Figs. 1.1 and 1.2; Relini *et al.*, 1998; Bell *et al.*, 2006, Katoh *et al.*, 2013).

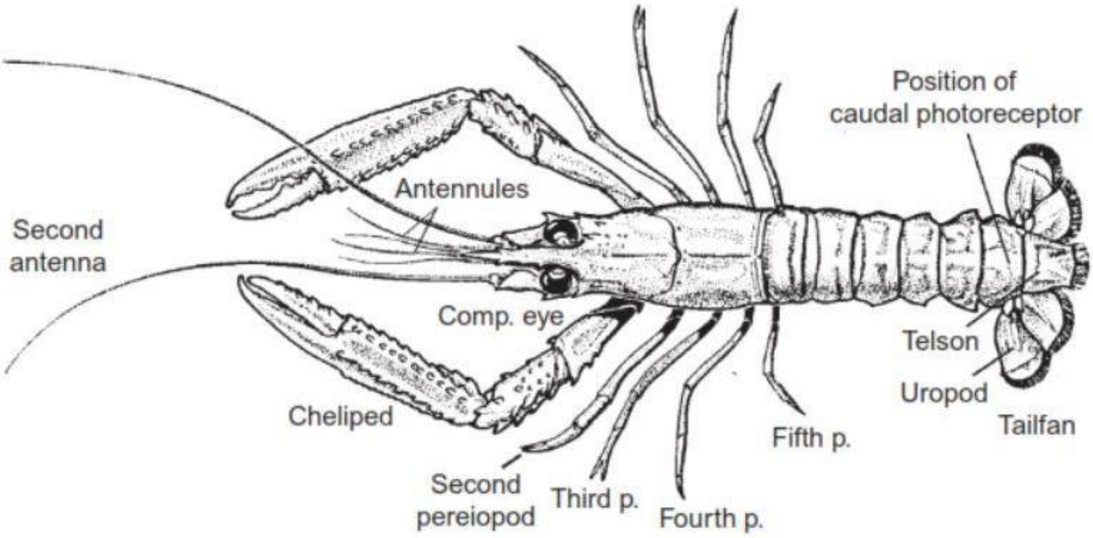


Figure 1.1. *Nephrops norvegicus* (Linnaeus, 1758). source: Katoh et al., 2013

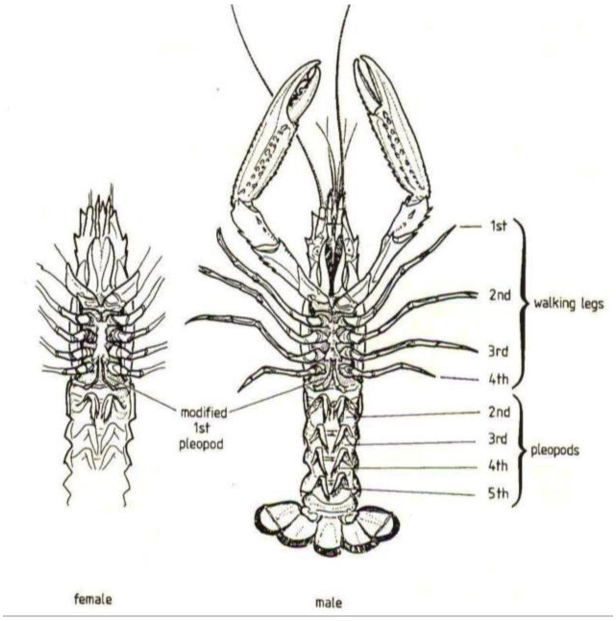


Figure 1.2. Ventral view of male and female of *Nephrops norvegicus*. Source: Howard, F.G. 1989.

As other members of the infraorder Astacidea, this species preferably walks on the bottom, while swimming mainly while it needs to escape (e.g. tail flipping; Katoh *et al.*, 2008). *N. norvegicus* (hereafter referred to by genus alone) is a member of the family Nephropidae which comprises other 40 similar species.

Nephrops generally lives at depths from 20 to 800 m on the continental shelves and slopes of the northeast Atlantic and the Mediterranean, spreading from Iceland and Norway in the north, to Morocco and Greece in the south (Fig. 1.3; Holthuis, 1996; Abello *et al.*, 2002). In the western Mediterranean, *Nephrops* populations are mainly located in deep waters on the continental slopes (300 to 600 m; Maynou *et al.*, 1998) or at 500–800 m depth East of Corsica and West of Sardinia (Abello *et al.*, 2002); while in the Adriatic Sea (central Mediterranean) the species was recorded over a wide depth range: from 50 m in the northern Adriatic down to 400 m in the southern basin (Froglia and Gramitto, 1981). *Nephrops* is restricted to marine waters (recorded only at salinities between 31.8 and 38.8) with relatively high oxygen concentration (between 5.9 and 9.4 mg O2/dm) and temperatures between 6.4 and 17.3 °C (Johnson *et al.*, 2013).

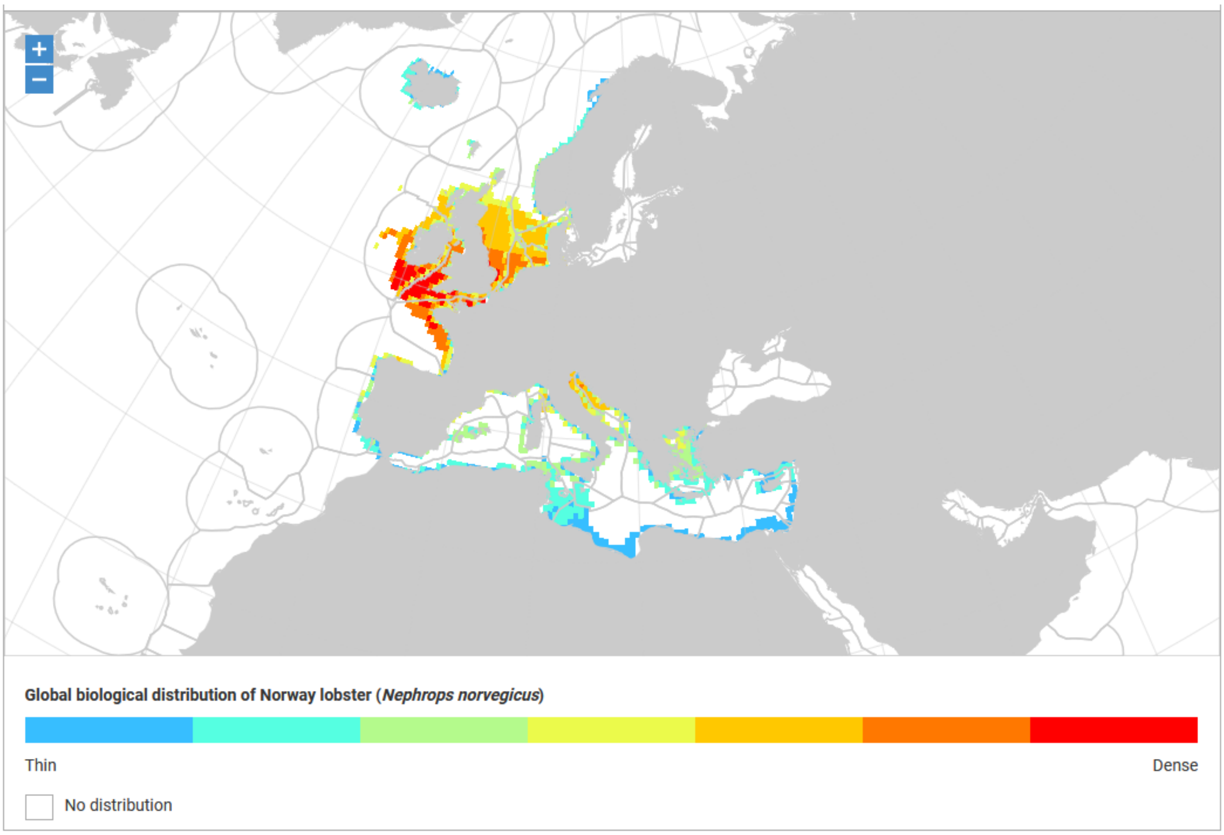
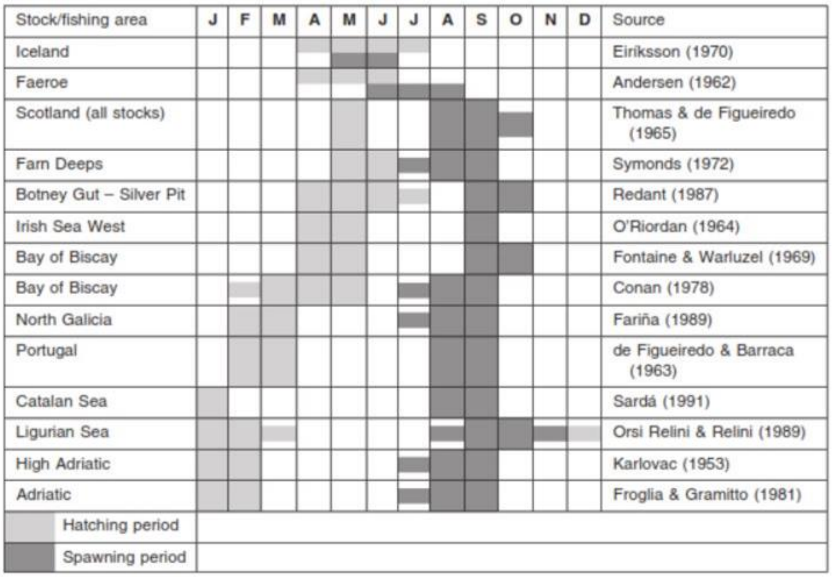


Figure 1.3. General distribution and density of *Nephrops norvegicus*. Source: https://www.seaaroundus.org

A close relationship between bottom sediment composition and *Nephrops* distribution is reported in literature (Farmer, 1975; Campbell *et al.*, 2009). Indeed, *Nephrops* preferably inhabits muddy seabed with medium-grained sediments (~ 40% of clay and silt; Bell *et al.*, 2006) where it can easily dig complex burrows system (Aguzzi and Sardà, 2008). The distribution of *Nephrops* depends also on larval supply modulated by existing local hydrodynamic regimes (Johnson *et al.*, 2013). Given that *Nephrops* is a sedentary species (Chapman and Rice, 1971), the recruits are hence related to larval dispersal from spawning to recruitment areas featuring distinct spatially segregated populations with little or no exchange between them (Bell *et al.*, 2006, Melaku Canu et al., 2020). O'Sullivan et al. (2014) finds in fact that larval connectivity between fishing grounds is dependent on variable seasonal conditions, geographical extension of an area and its proximity to other grounds.

Furthermore, *Nephrops* is a sedentary bottom-dweller that inhabits complex burrows constructed in muddy sediments within which it spends most of its lifetime (Abello *et al.*, 2002). In decapod crustaceans**,** shelter is an important ecological resource to avoid predation (e.g. Jennions *et al.*, 2003), to secure mating success (e.g. Atema and Steinbach, 2007) and to improve the feeding opportunities (e.g. Fero and Moore, 2008). Commonly, *Nephrops* leaves its burrows for feeding, moulting, breeding or to establish intraspecific interactions, remaining in their vicinity (Farmer, 1974; Aguzzi *et al.*, 2008; Sbragaglia *et al.*, 2017; Vigo *et al.*, 2021); the species displays aggressive behaviour over these limited resources that can culminate in fighting (Chapman and Rice, 1971). Indeed, laboratory studies provide evidence that adult males equally fed can establish a strong territoriality sustained by intraspecific encounters and consequent fighting; this behaviour supports the theory of a hierarchy of dominance (Sardà and Aguzzi, 2012; Sbragaglia *et al.*, 2017).

The species has separate sexes with internal fertilisation and visible external organs of reproduction; it commonly spawns once per year (Farmer, 1974). The periods of hatching and spawning, and the length of the incubation period vary with latitude; in populations where the reproduction cycle is annual, such as in the Mediterranean, mating occurs during the soft post-moult phase in winter/early spring (Bell *et al.*, 2006). Ovaries mature and eggs are spawned in summer (July – September; Figure 1.4), then they are incubated externally on the pleopods for 6 - 10 months depending on temperature (latitude) and habitat (i.e. 6 months in the Mediterranean; Farmer, 1974; Sardà, 1995); soon after spawning females carrying eggs by means of their pleopods hide in burrows until the larvae hatch in late winter (January - March; Fig. 1.4), after which they moult again (Relini *et al.*, 1998; Vrgoč *et al.*, 2004; Bell *et al.*, 2006). *Nephrops* is characterised by discontinuous growth occurring by means of moulting events, resulting in a relative size increment of 3–12% each (Bell *et al.*, 2006). Many studies recorded indeed considerably different values of increment at moult in relation to sex (Vrgoč *et al.*, 2004), maturity stage (Bailey and Chapman, 1983) or depending on the investigated population (Froglia and Gramitto, 1988). In the Mediterranean Sea adult females show only one growth event per year, in December – March, soon after hatching; the moulting peak for males is between June and September, while juveniles moult year-round (Gramitto, 1998).



Stock/fishing area	J	F	M	A	M	J	J	A	S	O	N	D	Source
Iceland				░	░ ▓	░ ▓	░						Eiríksson (1970)
Faeroe				░	░	░ ▓	▓	▓					Andersen (1962)
Scotland (all stocks)					░			▓	▓	▓			Thomas & de Figueiredo (1965)
Farn Deeps					░	░	▓	▓	▓				Symonds (1972)
Botney Gut – Silver Pit				░	░	░	░		▓	▓			Redant (1987)
Irish Sea West				░	░				▓				O'Riordan (1964)
Bay of Biscay				░	░				▓	▓			Fontaine & Warluzel (1969)
Bay of Biscay		░	░	░	░		▓	▓	▓				Conan (1978)
North Galicia		░	░				▓	▓	▓				Fariña (1989)
Portugal		░	░					▓	▓				de Figueiredo & Barraca (1963)
Catalan Sea	░							▓	▓				Sardá (1991)
Ligurian Sea	░	░	░					▓	▓	▓	▓	░	Orsi Relini & Relini (1989)
High Adriatic	░	░					▓	▓	▓				Karlovac (1953)
Adriatic	░	░					▓	▓	▓				Froglia & Gramitto (1981)
░ Hatching period													
▓ Spawning period													

Figure 1.4. *Nephrops* spawning and hatching periods. Source: Bell et al., 2006

The larval period of *Nephrops* is pelagic and planktonic, developing through three zoeal stages; the overall length of the larval period from hatching to metamorphosis depends on temperature (Farmer, 1975). Once hatched, larvae (Zoea stage 1) actively swim up the water column (positive phototaxis); the larger the larva is, the faster it can swim (Fig. 1.5; Powell & Eriksson, 2013).

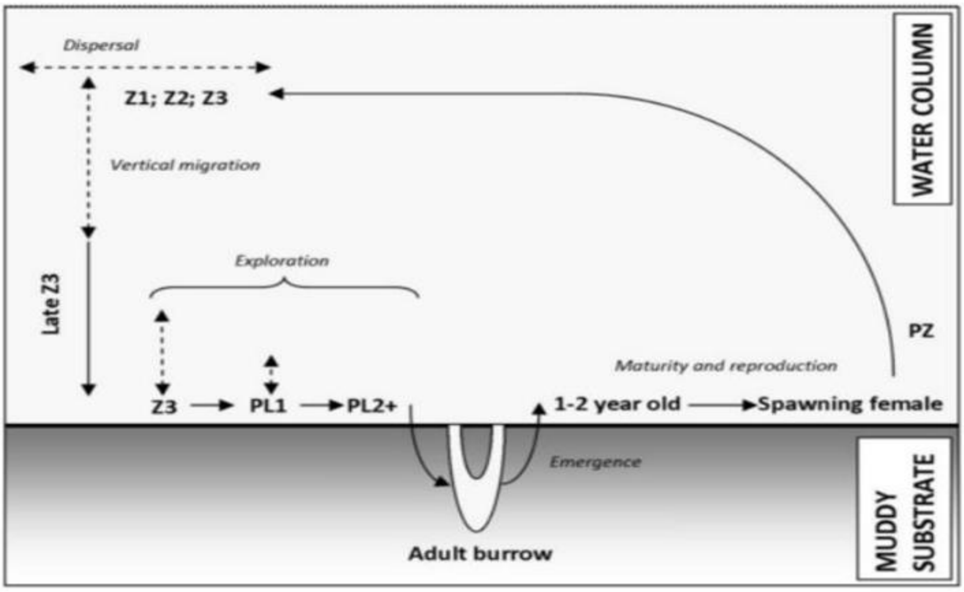


Figure 1.5. Habitat preference of *Nephrops* in relation to their life-cycle stage. PZ = pre-zoeal stage/eggs, Z1 - Z3 = zoeal stage 1 - 3, PL 1 - 2 = post-larval stage 1 and 2. Source: Powell & Eriksson (2013)

Subsequently, larvae perform daily vertical migrations, residing preferentially close to the pycnocline (Powell and Eriksson, 2013). During zoea stage 3, they become negatively phototaxic and move towards the bottom turning into demersal life; here they reside until metamorphosis into the post-larval juvenile benthic stage; at this point larvae still have some capability for vertical movement (Hill, 1990; Powell and Eriksson, 2013). At the end of the pelagic larval period, *Nephrops* individuals metamorphose and settle on the bottom; juveniles need muddy or silty-muddy substrata to survive (Farmer, 1975; Chapman, 1980; Hill, 1990). In the first post-larval stage (PL1; Fig. 1.5) they still have some ability to swim around and explore to find the best available substrate. At this point, post-larvae are morphologically similar to adults and start crawling, soon after they start inhabiting burrows occupied by adults within which they will reside for 1 - 2 years (Powell and Eriksson, 2013). Laboratory experiments showed that second stage post-larvae (PL2) are already capable of building small burrows (Eriksson and Baden, 1997) although in the wild they normally reside in adult burrows suggesting a chemotactic influence (Chapman and Rice, 1971). There is evidence in literature of young *Nephrops* leaving their burrows before the first year of age and their residence within an adult burrow appears to be linked not just to predator avoidance but also to feeding advantages (Tuck *et al.*, 2000; Powell and Eriksson, 2013). More frequent emergence events occurs in the second year of age up to sexual maturity (Powell and Eriksson, 2013).

Nephrops is commonly considered an active scavenger with a predominantly semi-fossorial foraging strategy, although cases of suspension feeding were reported for berried females (Loo *et al.*, 1993; Bell *et al.*, 2006); several authors reported non-selective feeding patterns for both sexes and diets heavily depending upon prey availability and abundance (Mytilineou *et al.*, 1998; Bell *et al.*, 2006; Johnson *et al.*, 2013). The opportunistic feeding behaviour is also proved by the prey diversity found in stomach contents for individuals from different areas, generally matching with the local benthic community composition (Wieczorek *et al.*, 2001). Indeed, studies in captivity showed that *Nephrops* prefers prey located close to its burrows (Aguzzi et al., 2009). In western Scotland, North Sea, Portugal and Mediterranean crustaceans, polychaetes and molluscs were identified as the bulk of *Nephrops*' diet (Bell et al., 2006). However, fish also represents a considerable contribution (Cristo & Cartes, 1998). The most common preys in the Northern-Central Adriatic Sea were for example found to be the brachyuran crabs Liocarcinus sp. and Goneplax rhomboides followed by fish and other benthic taxa (e.g. Polychaeta, Bivalvia etc; Zacchetti et al., submitted). Sinking discard from fishery activities could also be a source of food, thus this is also considered within ecosystem models (Angelini et al., 2016).

At high latitudes *Nephrops* is mainly predated by cod, *Gadus morhua*, and few other demersal fish species and cephalopods (Chapman, 1980; Coll *et al.*, 2006); for the Mediterranean Sea and the Bay of Biscay, information on predation is scarce and only few predators are known for *Nephrops* (Serrano *et al.*, 2003; Johnson *et al.*, 2013). This relatively low number of predators might be attributed to an interaction between the unavailability of the species owing to its burrowing behaviour and the large and spiny bodies of adult *Nephrops* (Johnson *et al.*, 2013). Furthermore, it was also observed, both in the field and in captivity, that *Nephrops* occasionally eats conspecifics, thus cannibalism could be as well considered as a trophic flow within ecosystem models (Baden et al., 1990; Sardà and Valladares, 1990; Cristo and Cartes, 1998; Angelini et al., 2016).

1.2 Exploitation of the resource and challenges in *Nephrops norvegicus* stock assessment

Commonly sold as Norway lobster, Dublin Bay prawn, scampi or langoustine, *Nephrops* is a commercially important species (Thorpe *et al.*, 2000), with global landing estimates of 75,900 tons reported in 2017 (Thorpe *et al.*, 2000; FAO, 2018). This crustacean is considered a delicious shellfish by chefs and consumers throughout Europe being one of the most important edible species in both the Eastern Atlantic region and the Mediterranean Sea (ICES, 2020). From 2007 to 2016, European's *Nephrops* landings were estimated to be worth over 281 million USD (Issifu et al 2022), while in 2017, the resource reached 278 million € (Marine Institute, 2017). The larger entire animals can indeed reach a price per kilogram equivalent to that of European lobster, *Homarus gammarus* (Sandberg *et al.*, 2004). The average European fishery production steadily increased in landings from less than 10,000 tons in 1950 to around 70,000 tons in 2007; from 2014 to 2019 the average production was about 52,000 tons, but a progressive decline in the following years was reported (FAO, 2021). Most of European *Nephrops* landings come from North-East Atlantic fisheries (i.e. United Kingdom, Irish, French, Danish, Iceland and Swedish) while Mediterranean ones (i.e. Italian, Spanish and Greek) contribute for almost 7 % (Ungfors *et al.*, 2013). The Mediterranean fishery production reached the highest landings from 1985 to 1997 with an average of about 7000 tons; subsequently the averaged landings decreased until 2005 with an estimate of about 5500 tons immediately followed by a further downfall until 2016; the average annual landings from 2016 to 2019 are about 2800 tons (FAO, 2021). In the Adriatic Sea, which is one of the main *Nephrops* fishing ground in the Mediterranean Sea, *Nephrops* ranked second among all the crustacean resources exploited in terms of abundance until 2014, however it showed a decreasing trend starting from 2005 (Vrgoč *et al.*, 2004, FAO, 2021). Focusing on the last ten years, the average landings for the Adriatic (Italy and Croatia) are about 1500 tonnes (FAO, 2021).

In European waters more than 95% of the total *Nephrops* landings are caught using single- or multi-rig trawlers (Ungfors *et al.*, 2013). However, in regions such as Western Scotland and the Swedish West Coast, the *Nephrops* proportion caught by creel fisheries is significant, accounting for up to a quarter of the total landings (Ungfors *et al.*, 2013). In the Adriatic Sea *Nephrops* is exploited prevalently by means of bottom trawlers (Vrgoč *et al.*, 2004; Ungfors *et al.*, 2013); only a small portion of the catches originates from creel fishery carried out along the Croatian channels, where access may be limited for trawlers due to shallow waters or legal restrictions (Morello *et al.*, 2009).

Estimating the abundance of a population is one of the main objectives of fisheries science aiming at the conservation and management of exploited resources (Shea, 1998). Population dynamics models for marine resources (“stock assessment models”) are widely used to estimate the current and historical status of marine populations, as well as to evaluate the likely consequences of alternative management actions (Hilborn and Walters, 2013). In the Mediterranean Sea, the main actors of the management of marine resources, included *Nephrops*, are: (i) the General Fisheries Commission for the Mediterranean (GFCM) of the Food and Agriculture Organization of the United Nations (FAO) and (ii) the Scientific, Technical and Economic Committee for Fisheries (STECF) of the European Commission (EC) (Carpi et al., 2017). Information about the distribution of marine species can essentially be derived from two main sources, namely, fishery-independent data (scientific surveys at sea) and fishery-dependent data (collection of data and sampling by observers on commercial vessels; Pennino *et al.*, 2016). One of the main sources of fisheries-dependent data for the EU countries are the National Programs implemented within the Data Collection Framework (DCF) designed to support the Common Fisheries Policy (CFP) through scientific advice (EU regulation 2017/1004). On the other hand, scientific trawling is generally the main fishery-independent source of information for the estimations of demersal species’ stocks; it can be used to provide biomass estimates by means of abundance indices and/or surface density data (i.e. the number of animals per unit of swept area; Briggs, 1987; Conan *et al.*, 1992; Fariña *et al.*, 1994; Maynou *et al.*, 1998). Scientific bottom trawl surveys provide a major source of additional fishery-independent information on abundance, species composition and basic biological data for the demersal communities (Smith, 1997; Cooper, 2006), and it is often one of the most technologically and economically feasible systems of sampling on shelves and slopes (Aguzzi and Sardà, 2008b, 2008a). These two sources may hence provide different types of information with additional details and their combination can improve stock assessments (Maunder, 2001). Therefore, a common objective of many population dynamics models is to adopt observed catches to estimate both population abundance and exploitation rates over time (Hilborn and Walters, 2013)

Unfortunately, rhythmic behaviour, which includes all motor acts involving a rhythmic repetition coupled with a cyclic variable, may deeply alter the general perception of population demography (Naylor, 1988; Nusbaum and Beenhakker, 2002). Given that most deep-water species undergo rhythmic displacement within the seabed, animals may be present or absent in a determined window of sampling, depending on their rhythmic status (i.e. active or inactive; Naylor, 2005). Therefore, when the timing of sampling is not considered, stock assessment results for certain species might be biased by behavioural rhythms (Naylor, 2005). In order to clarify the role that these deterministic variations play in the availability of fishery resources (e.g. Aréchiga and Rodriguez-

Sosa, 1997), biogeophysical cycles and behavioural rhythms should be identified and possibly measured in association with sampling (Naylor, 2010). In this context, the study of behavioural and physiological rhythms was introduced in the fishery management models of some commercially exploited species such as the Canadian sockeye salmon (*Onchorhynchus nerka*), the Atlantic oyster (*Crassostraea virginica*), the Australian spiny lobster (*Panulirus cygnus*) and *Nephrops* (Naylor, 2005).

Nephrops is an evident case of a commercially important species whose rhythmic emergence behaviour affects fishery, possibly biassing its stock assessment (e.g. Fariña *et al.*, 1994). This species shows high variability in catch rates over the diel cycle and this is related to fluctuations in numbers of individuals emerging from their burrows (Chapman *et al.*, 1972, 1975). Indeed, the bottom trawlers sweep the seabed capturing all animals that are performing emergence within the net mouth trajectory (Aguzzi and Company, 2010). Nevertheless, animals inside or at the entrance of their burrows easily avoid capture by retracting themselves when trawl nets approach (Rice and Chapman, 1971; Farmer, 1974; Main and Sangster, 1985, Aguzzi *et al.*, 2003). Several studies provided evidence that the time of day, the season, and the depth are important drivers of the capture rate and the size composition of catches (Hjellvik *et al.*, 2002; Naylor, 2005; Aguzzi and Sardà, 2008b). Hence a good estimation of the population density based on catch data can be theoretically produced only when samplings are conducted in identical circumstances (i.e. same timing and depth stratum; Petrakis et al., 2001; Benoît and Swain, 2003). Indeed, the maximum catch is obtained at different timings over the 24 hours according to the depth range, maintaining a very similar diel pattern across different geographic areas (Aguzzi and Sardà, 2008a, Aguzzi *et al.*, 2021). Field investigations revealed the presence of different emergence patterns depending on bathymetry: i) on the upper continental shelf (10-50 m), peaks of catch were recorded during the night and the emergence behaviour also appeared to be influenced by moonlight (Rice and Chapman, 1971; Chapman and Howard, 1979); ii) the maximum of emergence becomes crepuscular on the lower continental shelf (50 to 200 m), where high catches were observed around dawn and dusk (Chapman *et al.*, 1972, 1975; Farmer, 1975; Oakley, 1979); iii) the emergence pattern is fully diurnal on the upper slope at 200-430 m (Hillis, 1971; Aguzzi *et al.*, 2003). As a result of the burrowing behaviour, seasonal variations in sex ratio and size structures of commercial catches have been as well reported (Rice and Chapman, 1971; Chapman *et al.*, 1975; Sardà, 1995; Aguzzi *et al.*, 2004). In the Mediterranean Sea, catches decrease in autumn-winter shifting the sex ratio proportion towards males as females apparently suppress their diel cycle of emergence when berried (Aguzzi *et al.*, 2007). Currently, it is still unknown if the selective capturing of larger quantities of males compared to females could alter the overall population sex-ratio and consequently the behaviour, as demonstrated in other crustacean fisheries (e.g. van Son and Thiel, 2007). Besides, juveniles are captured in lower proportions than adults suggesting a different pattern of emergence (Gramitto, 1998; Aguzzi *et al.*, 2003). Both berried females and juveniles carry out opportunistic food retrieval collecting what is readily available in the close proximity of their burrow entrance (Aguzzi and Sardà, 2008a). Moreover, interindividual variability was recently observed in burrow emergence of *Nephrops* under simulated light cycles (Sbragaglia *et al.*, 2017). Emergence is also affected in a not fully clarified manner by other variables of environmental and demographic nature (e.g. cyclical seabed currents, hunger state, territorialism and density dependence; Katoh *et al.*, 2013). Taken together, these studies add other variables to the complex scenario of endogenous and exogenous control of individuals' emergence behaviour likely altering the perception of population dynamics. Hence, assessing the influence of sensory biology and behaviour of an organism of such economic importance as *Nephrops* should be considered crucial for a sustainable exploitation.

Following one of the most widely accepted definitions (see for example Waldman, 2005), a stock should be identified as an intraspecific group of randomly mating individuals with temporal or spatial integrity (Ihssen et al., 1981). Unfortunately, the term "stock" has been historically used to refer to both biological and management entities prompting confusion in the meaning of this term; indeed, the term is used here to refer to management stocks rather than biological stocks. A management stock (hereafter referred as stock) is thus defined as a group of conspecific individuals that are managed as a unit, separate from other groups of the same species (Wang, 2018). As mentioned above, *Nephrops* and similar species (e.g. *Metanephrops challengeri*, hereafter referred to by genus alone) show a very sedentary behaviour and only rarely migrate over distances longer than a few hundred metres (Jensen, 1965; Chapman, 1982; Vigo *et al.*, 2021). Therefore, within areas that are considered to be inhabited by the same stock unit, the distribution of this demersal species can be very heterogeneous showing growth and morphometrical differences inside the same area (Maynou and Sardà, 1997). For example, in European waters Bell *et al.* (2006) identified more than 30 different *Nephrops* populations, which are all physically isolated from each other, while Tuck (2009) shows that *Metanephrops* around New Zealand is divided into five distinct main and other smaller populations. Heterogeneity in distribution is also present within smaller areas, giving rise to smaller subpopulations (Chapman and Bailey, 1987). Within a stock unit, a population may thus be divided into smaller "stocklets" (i.e. subpopulations), with different densities, size and sex compositions, and also different biological features (growth rate, size at first maturity, etc.; Maynou and Sardà, 1997; Bell *et al.*, 2006). The high variability among subpopulations is therefore one of the main problems for the analytical stock assessments of *Nephrops*; together with the difficulties in directly estimating recruitment (Wahle, 2003). Furthermore, the main issue is the lack of reliable ageing methods (Bell *et al.*, 2012). In fact, a direct determination of age in *Nephrops* is not routinely possible so far, although the demonstration of consistent banding by Kilada *et al.* (2012) is promising. On the other hand, the development of size modes interpreted as age cohorts allow inferences on growth patterns from size-frequency data (Dobby & Hillary, 2008). However, unambiguous identification of age cohorts is often problematic, particularly after sexual maturity when the variability of growth pattern is high meaning that age-classes are not clearly separable in terms of size modes (Bell *et al.*, 2006). Although growth is a discontinuous process in *Nephrops*, for the purposes of stock assessment, continuous growth curves such as the Von Bertalanffy growth function are generally used as a mathematically convenient description of the relationship between average size and age (Ogle and Isermann, 2017). The main parameters of the function are L_∞, the mean size that an animal can reach over infinite time, and K, the rate at which this size is approached; a third parameter t_0, the theoretical age at zero size, scales the growth curve to absolute rather than relative ages and cannot always be estimated. Mytilineou et al. (1998) and Ulmestrand and Eggert (2001) discussed in detail the difficulties in estimating *Nephrops* growth by deriving Von Bertalanffy's functions from mark-recapture methods and/or length cohort analysis (LCA); basically, these studies indicate that a large variability in estimates may be generated by factors as the months included in the analysis, the precise stations in which sampling was conducted, or the calculation method. As an example, the Figure 1.6 reports the growth curves for some Atlantic and Mediterranean "stocklets" by sex underling their wide variations.

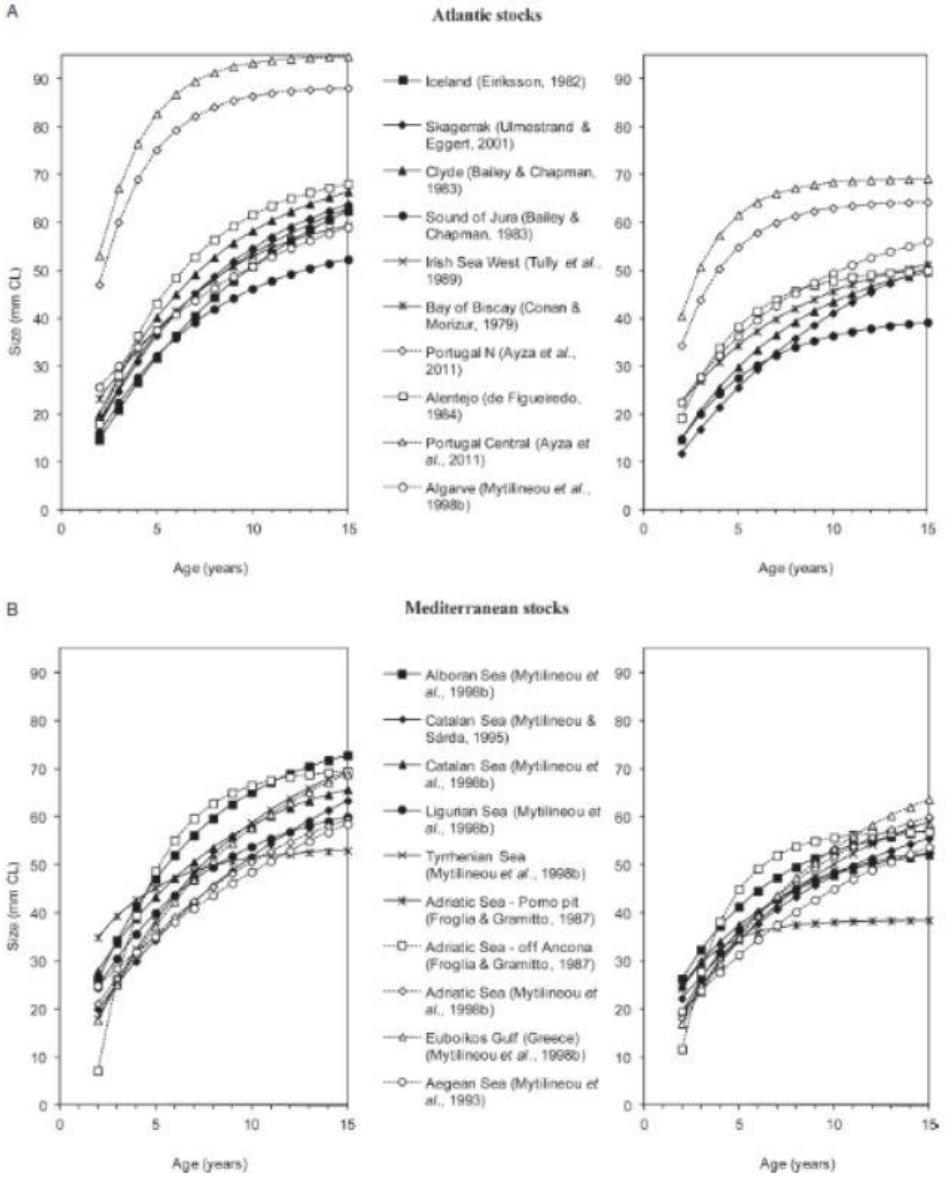


Figure 1.6 A) Growth curves of male (left) and female (right) Nephrops – Atlantic "stocklets". (B) Growth curves of males (left) and female (right) Nephrops – Mediterranean "stocklets". Source: Bell et al., 2006

In addition, as above mentioned, differences in growth rates can also occur over quite small spatial scales. Within the same biological population, there may be major differences in the size structure and growth parameters of subpopulations of *Nephrops* inhabiting smaller areas with different demographic characteristics (Figueiredo, 1984; Tully and Hillis, 1995; Tuck *et al.*, 1997; Thompson *et al.*, 1998). For example, in the Clyde (West Scotland), the K values for male *Nephrops* were found to range between 0.16 and 0.22 year, and the values of L∞ between 45.5 and 65.5 mm CL (Tuck *et al.,* 1997). The exact reasons for the variability in growth rates are difficult to establish, since they are likely to be result of the combined effect of several drivers (temperature, sediment particle size, food availability, population density, fishing pressure, etc.), each possibly having different interactive effects (Tuck *et al.*, 1997; Sánchez Lizaso et al., 2000; Bell et al. 2006).

As reported in Bailey (1986), the use of growth parameters such as Von Bertalanffy's L∞ for *Nephrops* or similar species, do not account for the density-dependent suppression of growth proper of some 'stocklets'. Tuck *et al.* (1997) instead examined the variability of L∞/density at a small spatial scale (southern Clyde), observing smaller L∞ at higher densities. As reported in Bell *et al.* (2006), the density-dependent suppression of growth partially explains size distributions of *Nephrops* at broad scales (e.g. Functional units in ICES regions, FU). In general, fisheries are well known to modify the size distributions of target species, thus probably influencing the size–density relationships, as shown for *Nephrops* in some Mediterranean cases (Abello et al., 2002, Dimech et al., 2012). At broad scales, different size modes for *Nephrops* stocks could also be partially explained by growth suppression due to competition and/or recruitment effects (Johnson et al.; 2013).

Overall, the assessment of *Nephrops* is thus fraught by several difficulties, from their burrowing behaviour, that results in different catch patterns, to the marked sexual dimorphism, the uncertainty about growth, and the lack of reliable age-determination methods.

1.3 Presence of different subpopulations of *Nephrops norvegicus* within the Adriatic Sea

The Adriatic Sea represents an ideal area for the settlement of *Nephrops* due to the presence of muddy (silty-clay) grounds at depths from around 30 m to over 400 m (Artegiani *et al.*, 1979; Wieczorek *et al.*, 2001). The main fishing grounds for this species were observed at depths of approximately 70 m off Ancona, around 220 m in the Pomo/Jabuka Pits and approximately 80–90 m in the Velebit Channel, Kvarner and Kvarneriç regions along the Croatian coast (Karlovac, 1953; Crnkovic, 1969; Froglia and Gramitto, 1981, 1986, 1988; IMBC UMBSM, 1994; Froglia *et al.*, 1997; Colloca *et al.*, 2015; Russo *et al.*, 2018; Fig 1.6).

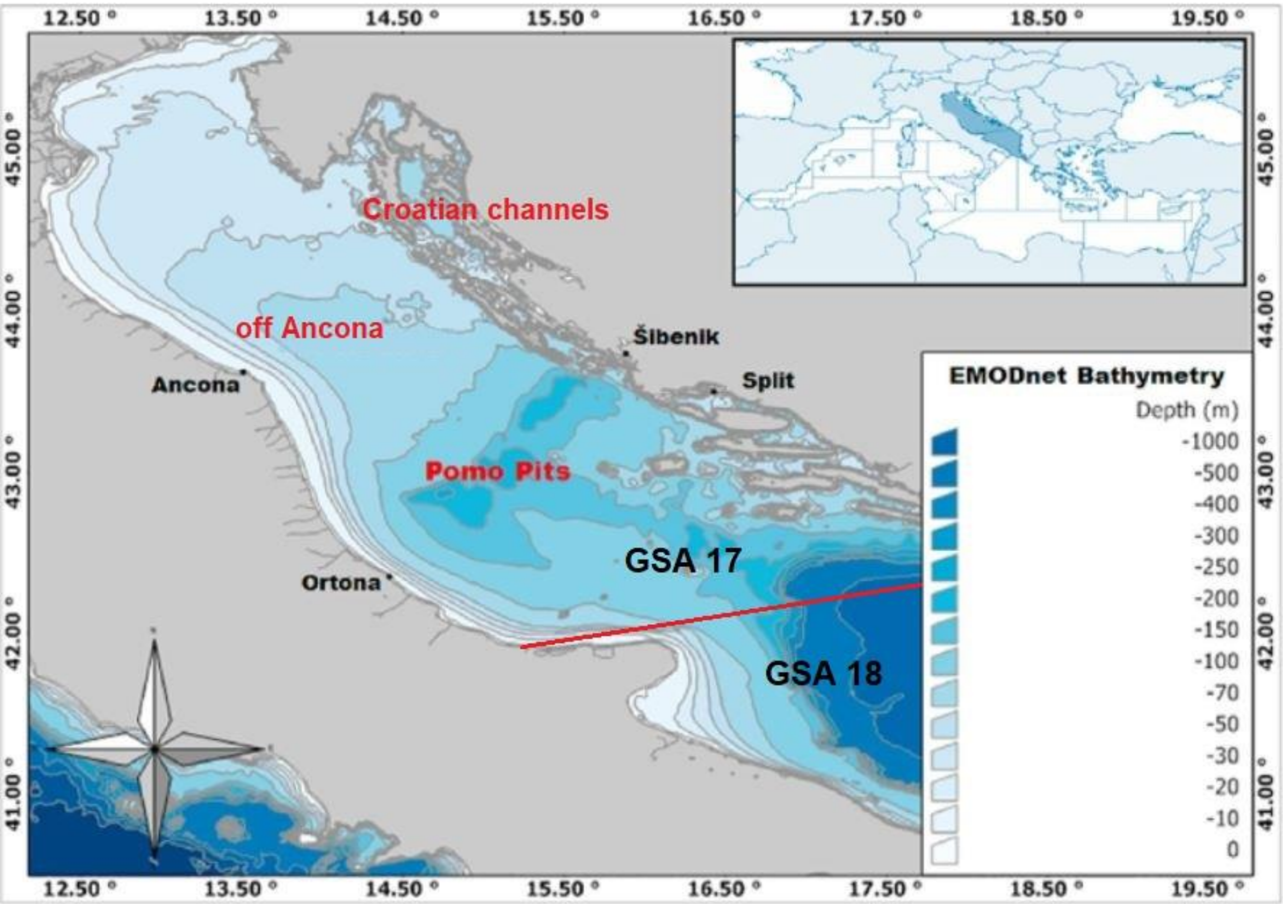


Figure 1.6 In the up-right rectangle the position of the Adriatic Sea within different Mediterranean FAO-GFCM GSAs (geographical sub-areas) is evidenced (the Adriatic Sea corresponds to GSA17 + GSA18). The main map shows the position of the three meso-Adriatic depressions well known as “Pomo Pits”, the “off Ancona” ground located along the Italian coast, and the “Croatian channels” (including Velebit Channel, Kvarner and Kvarneriç region; see Karlovac, 1953 and Crnkovic, 1969 for details) located along the Croatian coast. The red line divided the GSA 17 from the GSA 18. Bathymetry is from https://www.emodnet-bathymetry.eu.

In the Mediterranean Sea, resources are managed at a Geographical Sub-Area (GSA) scale and the Northern-Central Adriatic basin corresponds to GSA 17, while GSA 18 refers to the Southern Adriatic Sea (Cataudella and Spagnolo, 2011; Fig. 1.6). Despite the fact that FAO-GFCM and STECF have repeatedly recognized the limits of a GSA-based approach, the management advice is currently provided at GSA level (STECF, 2014). The GSA definition is based on management convenience rather than biological inference (Smedbol and Stephenson, 2001). Indeed, several marine species appear to have more complex stock structure than is recognized, and management units may include multiple subpopulations with different biological features rather than a single discrete population (Stephenson, 1999). Furthermore, fleets operate without considering the spatial structure defined by the FAO-GFCM GSAs, which instead represent the basis for the official commercial data (i.e. DCF) and, consequently, for stock assessment. This appears to be exactly the case of the Pomo/Jabuka Pits in the central Adriatic Sea. According to the most recent STECF report (STECF, 2020) treating and assessing the *Nephrops* population at a GSA or joint GSA level may be questionable and could lead to an inaccurate and imprecise evaluation of the status of the resource (STECF 2016; Carpi *et al.*, 2017). The lack of spatially explicit catch data further complicates the assessment efforts as Italian southern Adriatic trawl fleets (GSA 18) often fish in the Pomo/Jabuka Pits (GSA 17) and land in GSA 18 (Russo *et al.*, 2018). Hence, to appropriately account for complex patterns of fleet behaviour and/or different aspects of species behaviour and biology, the assessment of the status of *Nephrops* stock within the Adriatic Sea need to be based on data collected at a higher spatial resolution than the GSA (Carpi *et al.*, 2017; Russo *et al.*, 2018)

The Mediterranean Sensitive Habitats project (MEDISEH) identified several spawning grounds within the Croatian channels, in the “off Ancona” ground and along the Italian coast, and a main recruitment area located in the Pomo/Jabuka Pits, which therefore is of particular interest, as it was identified both as a recruitment area and a spawning ground (i.e. both spawners and recruits were collected; MEDISEH, 2013). This is also confirmed by a recent connectivity study from Melaku Canu *et al.* (2020), which analyses 20 potential spawning areas within the Adriatic basin and suggests the presence of at least three distinct subpopulations inhabiting the Pomo/Jabuka Pits, the “off Ancona” ground and the Croatian channels. The presence of discontinuously distributed *Nephrops*’ groups is supported by numerous studies which also provided evidence of difference density ranges and growth patterns within the Adriatic Sea (Karlovac, 1953; Froglia and Gramitto, 1981, 1988; IMBC UMBSM, 1994; Morello *et al.*, 2009; Carpi *et al.*, 2017; Angelini *et al.*, 2020). The Pomo/Jabuka Pits is an area characterised by particular oceanographic conditions (Marini et al., 2016) and it is occupied by a very dense subpopulation of small animals with slow growth rates and small size at first maturity (Froglia and Gramitto, 1981, 1988; IMBC UMBSM, 1994; Angelini *et al.*, 2020, Fig. 1.7).

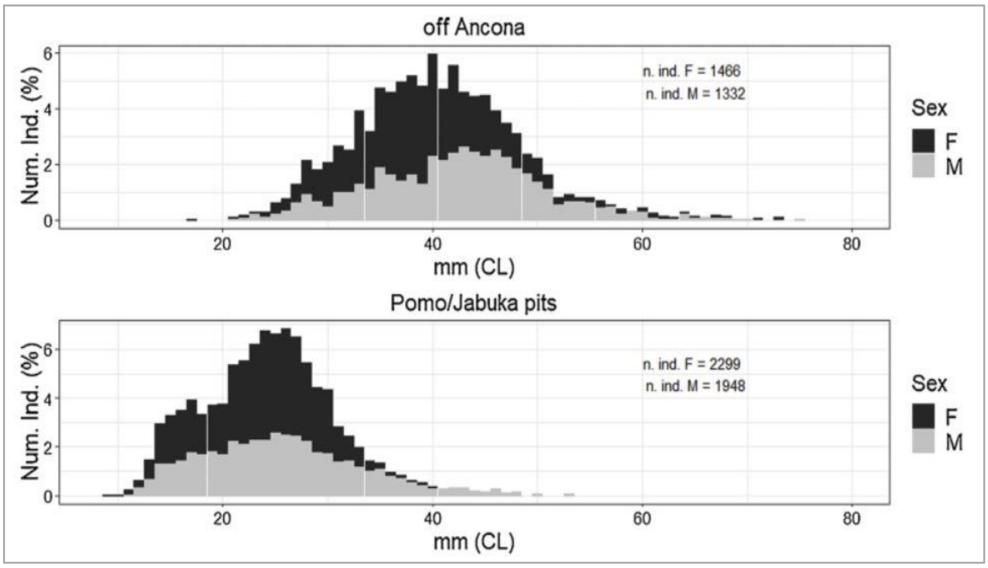


Figure 1.7 Length frequency distribution of Nephrops divided by sex (grey males, black). Source: Angelini et al., 2020

For this reason, the individuals inhabiting the Pomo/Jabuka Pits should be considered as a separate subpopulation from those settled in "off Ancona" ground (Froglia and Gramitto, 1988) and along the Croatian channels (Vrgoč *et al.*, 2004). Notwithstanding these evidence, genetic analysis did not confirm any differences between these subpopulations (Mantovani and Scali, 1992; Guarniero *et al.*, 2007, Martinelli et al., 2021); thus environmental drivers (Mantovani and Scali, 1992) and density-dependent effects (Wieczorek *et al.*, 2001) could probably be the factors responsible for these diversities.

1.4 Study area: the Pomo/Jabuka Pits

The Central Adriatic is a transition zone between the shallow Northern Adriatic shelf and the deep Southern Adriatic basin (Artegiani et al., 1997). It is characterised by three pits collectively known as Pomo or Jabuka Pits (in Italian and Croatian language respectively) bounded by the 200 m bathymetry, with a maximum depth of about 270 m (Figure 1.6; https://www.emodnet-bathymetry.eu). This complex morphology provides sinks where cold dense water is trapped for one or more years strongly impacting sea-floor sediments and affecting nutrient and organic matter export to the Southern Adriatic deep sea (Canals *et al.*, 2006, 2009). Dense water masses are generated in the northern Adriatic Sea due to the strong, cold and dry winter NE winds (Bora) that blow on the northern shelf (Gačić *et al.*, 2001). These katabatic winds combined with the evaporation contribute to the formation of the densest water in the whole Mediterranean during January and February (temperature ~ 11°C, salinity ~ 38.5, density ~ 29.6; Hendershott and Rizzoli, 1976; Orlic *et al.*, 1992; Malanotte-Rizzoli *et al.*, 1997; Vilibić and Supić, 2005). This water mass, named North Adriatic Dense Water (NAdDW), exerts a strong impact on Central Adriatic oceanography and it has been estimated to account for up to 20% of the total Adriatic deep water volume (Vilibic and Orli, 2002). Deep water cascading events that are dense enough to flow close to the sea floor may affect directly and indirectly the distribution and abundance of sessile deep-water benthic organisms (Canals *et al.*, 2006). The Adriatic dense water cascading may convey a surplus of nutrition to the sea-bottom by flushing the seafloor and exporting and/or re-suspending organic matter deeper than usual; this modification in the nutrient cycle has an impact on local biodiversity and the trophic status of benthic communities (Taviani *et al.*, 2016).

The Pomo/Jabuka Pits present a seabed mainly composed by fine muddy substrates (Trincardi *et al.*, 2014) which form an ideal habitat for settlement of *Nephrops* populations as well as other demersal crustacean species such as *Solenocera membranacea* (Risso, 1816), *Calocaris macandreae* (Bell, 1846) (Marrs *et al.*, 1996; ICES, 2008; Martinelli *et al.*, 2021). A crustacean taxon of particular ecological interest for the area is represented by *Munida spp.*: *Munida intermedia* (A. Milne-Edwards & Bouvier, 1899), the species that originally resided in the area, in a few years starting from 2000, was totally replaced by another squat lobster species, *Munida speciosa* (von Martens, 1878) (Froglia et al., 2010, 2017). *Munida spp.* it's not the only macrobenthic organism of ecological importance, sea pen species as *Funiculina quadrangularis* were observed within the the Pomo/Jabuka Pits (Martinelli et al., 2013); these are considered species very sensitive to physical disturbance (e.g. trawling activities) and hydrodynamic conditions (Greathead et al., 2007; Salvalaggio et al. 2016). Historically, *Parapenaeus longirostris* (Lucas, 1846) were recorded between 200 and 400 m depth in the Adriatic basin including the Pomo/Jabuka Pits (Froglia, 1982; Kolitari et al., 2014). In the last decades, a significant increment of the biomass of this crustacean decapod was observed in the whole Mediterranean Sea, such an increment was related to its warm-temperate affinity promoted by the warming process that occurred since the 1950s within the Mediterranean basin (Vargas-Yánez et al. 2009, Skliris et al., 2012; Colloca et al., 2014). Furthermore, this area was also identified as a nursery ground for the European hake, *Merluccius merluccius* (Linnaeus, 1758), which is another important commercial demersal species for the Adriatic Sea (Colloca *et al.*, 2013; Druon *et al.*, 2015).

Historically, the Pomo/Jabuka Pits was heavily exploited by bottom trawling of both Italian and Croatian fleets (Cardinale *et al.*, 2008), the harmful impact of fishery caused habitat degradation and overexploitation of juveniles leading such region to be identified as vulnerable marine ecosystem (VME; De Juan and Lleonart, 2010). The Pomo/Jabuka Pits was subjected to several discussions regarding the possibility of establishing an area closed to fisheries in order to protect VMEs and essential fish habitats (EFH) for demersal stocks such as European hake and *Nephrops* (AdriaMed, 2008; De Juan and Lleonart, 2010; Fanelli et al., 2021). With the aim to improve the exploitation and conservation of these demersal stocks the first partial closure of trawling activities was approved in July 2015 (D.M. 03/07/2015 and D.M. 20/07/2016; N.N. 20/07/2015 and N.N. 22/07/2016);

subsequently several changes in the management measures occurred in terms of spatial and temporal limits of the fishery restrictions (D.M. 19/10/2016; D.D. 7/12/2016; N.N. 17/05/2017). Finally since 2017 the Pomo/Jabuka Pits area was officially recognized as Fishery Restricted Area (FRA) that means a geographically-defined zone in which some specific fishing activities are temporarily banned or restricted in order to improve the exploitation and conservation of demersal stocks (GFCM, 2017; MIPAAF, 2017; EU, 2019); it was then identified a fishery ban zone (A) and other two buffer zones where the trawling activity is limited to a specific number of authorised vessels and fishing days (B and C; GFCM, 2017).

In this study, the spatial extent identified as "Pomo" includes the FRA zones A, B and C plus an additional external buffer zone that corresponds approximately to what is envisaged as being the spatial domain of *Nephrops* stocklet of Pomo/Jabuka Pits (Fig. 1.8). Further justification for the inclusion of this buffer zone in this study was due to the spatial dimension used to provide both fishery-dependent and fishery-independent data helping in the estimation of landings and survey indices.

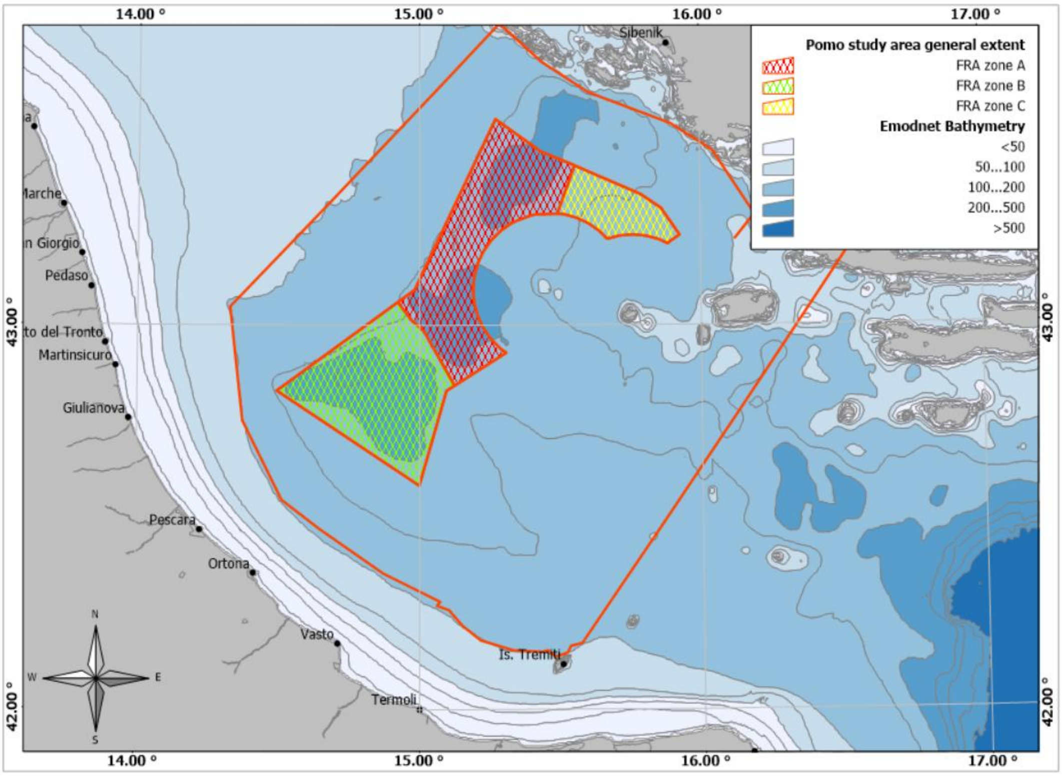


Figure 1.8 The study area of "Pomo". The map shows the boundaries of the general spatial extent identified as "Pomo", with the inner red polygons correspond to the latest management measures of the Pomo/jabuka Pits FRA (fishery ban zone (A) and the two buffer zones (B and C); GFCM, 2017; MIPAAF, 2017; EU, 2019). Bathymetry is from https://www.emodnet-bathymetry.eu.

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2. Aim of the study and objectives

Currently, the institutions officially in charge to give a scientific advice on the state of exploitation of the Mediterranean's stocks (i.e. GFCM & STECF) have not found yet an agreement on an assessment of *N. norvegicus* stock valid for the Adriatic Sea (GSA 17 e GSA 18 combined). The main challenges of this assessment are represented by: (i) factors related to the biology and behaviour of the species itself, as sexual dimorphism, discontinuity in growth, lack of reliable age-determination methods, burrowing behaviour and emergence patterns which alter its availability to the fisheries; (ii) presence of smaller "stocklets" featured by subpopulations with different densities, sizes, sex compositions and biological traits within a large spatial scale (e.g. in the Pomo Pits within GSA 17); (iii) the spatial patterns of data collection. Furthermore, as demonstrated by several studies, alterations of the bottom environmental parameters as temperature, salinity and oxygen concentration could influence the spatiotemporal distribution and the recruitment rate of *N. norvegicus*, hence impacting on its abundance. Additionally, changes over time and space of management strategies (e.g. implementation of fishery ban zones) can make even more complex the task of assessing the local abundance of a sedentary resource.

The aim of the present study was to perform for the first time a thorough evaluation of the status of the *Nephrops norvegicus* population inhabiting the Central Adriatic Sea and to evaluate possible effects of the management measures recently implemented in the area. The Central Adriatic Sea, and in particular the Pomo or Jabuka Pits, represents indeed an interesting case study due to: (i) the oceanographic and geophysical peculiarities of the Pits leading to occasional water mass renewals; (ii) the presence of a *N. norvegicus* population characterised by very small animals with high density, slow growth rates, and small size at first maturity; (iii) the activity of two different trawl fleets, the Italian and the Croatian ones; (iv) the establishment of a Fishery Restricted Area (FRA) aimed at the conservation of marine ecosystems and essential fish habitats since 2017 (e.g. the Pomo/Jabuka Pits also represent a nursery area for the European hake *Merluccius merluccius*).

The available datasets useful to assess *N. norvegicus* within the Pomo/Jabuka Pits consists in fishery-independent data from scientific surveys carried out specifically to monitor this important area of the Adriatic Sea and official commercial catch data collected within the Italian and Croatian Data Collection Framework (DCF) for the Northern and Central Adriatic Sea. Therefore, two scientific trawl surveys specifically targeting *N. norvegicus* are annually carried out by CNR IRBIM within the Pomo/Jabuka FRA: the spring/summer "UWTV" survey conducted over the entire extent of the Pomo Pits region since 2012 and the autumnal "ScamPo" survey carried on since 2015 over the Italian side of the Pits area. Furthermore, during the "UWTV" survey, seabed footage is collected and used to derive absolute numbers of individuals through a series of assumptions. Such fishery-independent data, which have never been considered before for stock assessment purposes, and commercial ones specifically tuned for the Pomo/Jabuka Pits area spatial domain, can be thus combined in a population dynamic model to better describe the demography of *N. norvegicus* inhabiting the Central Adriatic Sea.

Therefore, within this work, a lot of effort has been put in the treatment of collected data to be used to feed the stock assessment model, in order to obtain the most reliable abundance estimates from both fishery-dependent and -independent approaches: first, the estimated biomass and density indicators were refined with a revision of some assumptions (particularly for density indices estimated from the video counting analysis), as well as through the development of statistical models able to predict these indicators considering both the effect of environmental variables and fishery management measured implemented in the study area. Then, the spatio-temporal scale of these refined data, together with the historical ones, were re-arranged on the basis of the emergence patterns and the extension of the study area that encompasses a specific "stocklet".

To overcome all the above mentioned issues, within this study an explicit length-structured, sex- and fleet-based integrated assessment method, directly using length data in the form of size-transition matrices, is developed. Through CASAL software, a Bayesian length-based stock assessment model is indeed adopted to combine commercial data with all the information collected during fishery-independent surveys. The overarching goal is to build a framework to develop an accurate stock assessment model through a novel approach that could be later extended to the rest of the Adriatic Sea.

3. The UnderWater TeleVision (UWTV) survey

As described in section 1.3, *Nephrops* fisheries exhibit strong temporal patterns in catch rates linked to the biology and behaviour of the species. This makes traditional trawl surveys (e.g. MEDITS; Bertrand *et al.*, 2002) not fully reliable while assessing this species, because catch rates are not necessarily indicative of real abundance at sea (Scientific, 2016; Carpi *et al.*, 2017).

Currently, no methods are available to directly and accurately age *Nephrops* (Sheridan *et al.*, 2015); in addition, indirect age estimation, although possible, is difficult in many stocks due to the lack of variability in year class strength and contrast in the observed length frequency distributions (Bell *et al.*, 2006). These issues led to the use of towed underwater camera (UnderWater TeleVision; UWTV) surveys as an alternative approach to assess stock status and provide management advice in several European countries (Leocádio *et al.*, 2018). Basically, the UWTV standard design consists in a camera system mounted on a sledge towed by a vessel by means of an armoured cable that provides towing capabilities and includes power/coaxial line, enabling researchers on board the vessel to visually assess a known surface area of seabed (Leocádio *et al.*, 2018).

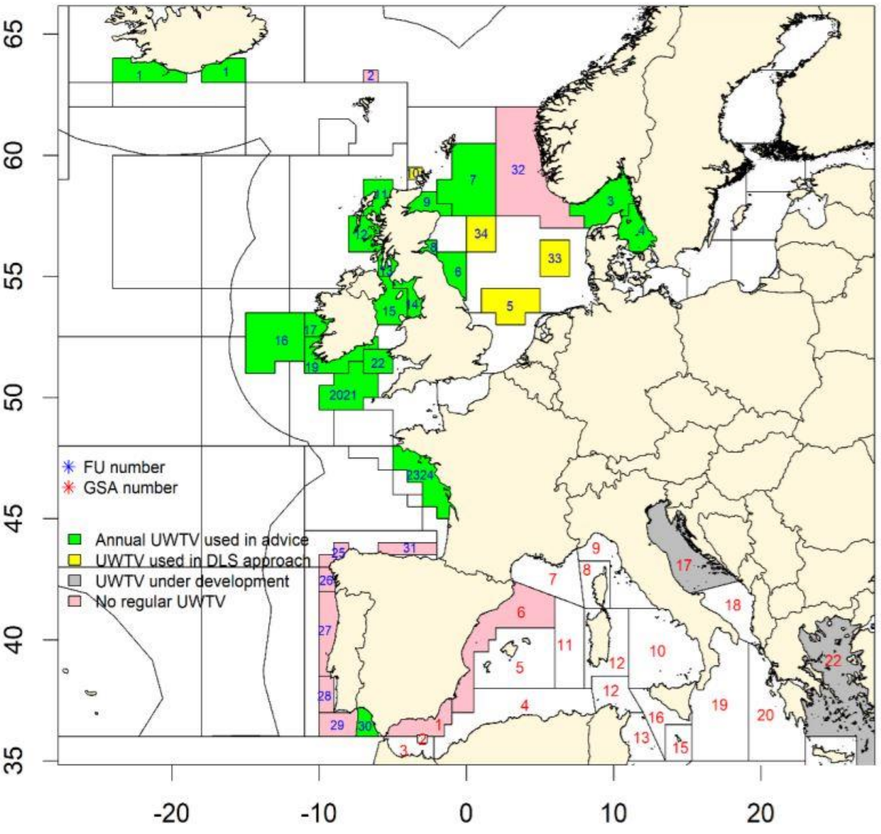

Figure 3.1 Nephrops UWTV survey areas and use in stock assessment (FU: Functional Unit, GSA: Geographical Sub Area, DLS: Data Limited Stock). Source: ICES, 2021

Historically during the 1990s, scientists and engineers at the Marine Laboratory (Aberdeen, Scotland) carried out the first UWTV surveys on the east coast of Scotland to compare the relationship between the number of *Nephrops* burrow systems observed in the collected video footage and the population abundance estimates obtained from analytical stock assessments (Leocàdio et al., 2018). According to studies by Farmer (1974) and Rice and Chapman (1971) about field assessment of *Nephrops'* diurnal activity, there were evidence to assume that one animal inhabited one burrow complex. Therefore, theoretically, the number of animals in a specific area could be calculated by counting the number of complexes based on specific morphological features over a known surface area and raising this value to the known area inhabited by *Nephrops* (Chapman *et al.*, 1975; ICES, 2017). The derived burrow densities (n/m) can be then used as an index of population abundance (Maynou *et al.*, 1998; Aguzzi *et al.*, 2004; Sala, 2018). Thus by 2006, all the major Scottish *Nephrops* stocks were assessed based on UWTV surveys providing *Nephrops* abundance by functional unit within the network of the International Council for the Exploration of the Sea (ICES), an intergovernmental marine science organisation; later in 2007, the first ICES *Nephrops* UWTV workshop on the use of UWTV surveys for determining abundance in *Nephrops* stocks throughout European waters (WKNEPHTV) took place (ICES, 2007). From here on, various ICES Study Group on *Nephrops* Surveys (SGNEPS) were arranged discussing if the UWTV method was the best suited to provide an index of population abundance, in accordance with the various assumptions on which it is based (ICES, 2008, 2009a, 2010, 2012a). These meetings did not only take into account the uncertainties associated with UWTV surveys but they also discussed the production of standard protocols for the processing of UWTV survey data, and the relative merits of the various survey designs and technological advancements made by different institutes (ICES, 2009b, 2012b). Finally in 2013, the Working Group on *Nephrops* Surveys (WGNEPS) was established and it is currently the coordination expert group for *Nephrops* UWTV and trawl surveys in Europe (ICES, 2013). Thus WGNEPS provides well established and documented advices on the basic survey design, quality control measures, core equipment, and data work-up; such information allows institutes to extent their survey programs and to investigate uncertainties associated with the UWTV approach in an attempt to improve the quality and use of the collected data (Petitgas et al., 2017; Leocàdio et al., 2018; Dobby et al., 2021). Currently, UWTV surveys are used to provide population estimates for *Nephrops,* in various ICES functional units (FUs) and on an experimental basis in few geographical subareas (GASs) in the Mediterranean (Fig. 3.1; ICES, 2021). In most FUs the UWTV survey is mandatory for management advice purposes, while in GSAs it is used to provide additional information on the population (Dobby et al., 2021).

After some trials in 1994 and 2004 (Froglia *et al.*, 1997; Morello *et al.*, 2007), in 2009 CNR-IRBIM of Ancona, in collaboration with IOF of Split (Croatia) and under the auspices of the FAO – AdriaMed (Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea) regional project, started a series of UWTV surveys in the Pomo/Jabuka Pits area (Martinelli *et al.*, 2013). Also thanks to the Italian National Flagship Program RITMARE, in 2013 the UWTV equipment was completely renewed and enriched with new sensors allowing the collection of corollary environmental parameters (Martinelli *et al.*, 2016); therefore ecological datasets can be produced and used in the context of an ecosystem approach to fisheries management (Tuck, 2009; Angelini *et al.*, 2016). Except for 2011 and 2018, a spring survey has been carried out yearly from 2009 to 2019 in the study area on board the R/V Dallaporta (LOA 35.30 m, 258 GT, 1100 193 HP). The UWTV camera (Kongsberg Simrad OE 1364 colour camera) was mounted on a sledge towed on the seabed at a speed of 1 knot with the field of view of the camera fixed at 80 cm width; the position of the sledge at each minute was recorded by means of a custom-made datalogger synchronised with the camera deck unit (Martinelli *et al.*, 2013). The number of UWTV stations was designed proportionally to the surface area for a total of 60 planned ones (Fig. 3.2), and each of them entailed an effective towing time of almost 20 minutes (Martinelli *et al.*, 2013). Unfortunately, often the number of achieved stations (compared to the planned ones) was reduced because of bad weather conditions and limited available ship time (Martinelli *et al.*, 2019).

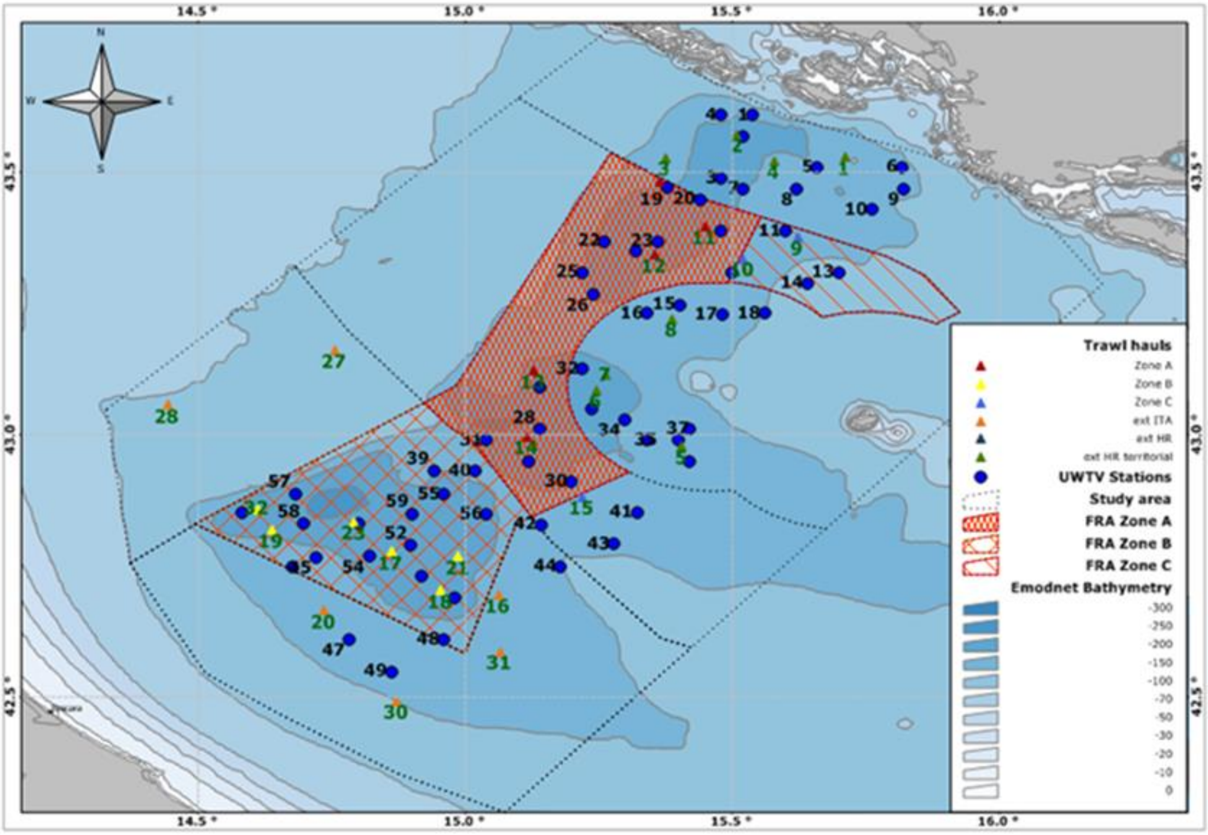

Figure 3.2 The UWTV survey design. With blue points are indicated the camera stations, while with coloured triangles the fishing hauls; the red polygons are the boundaries of the Fishery Restricted Area of Pomo/Jabuka Pits (GFCM, 2017);bathymetry is from *https://www.emodnet-bathymetry.eu*

3.1 Lin’s CCC analysis

Presented at WGNEPS 2019: Aristegui-Ezquibela, M., Burgos, C., **Chiarini, M.**, Cvitanić, R., del Rio, J., Doyle, J., Fifas, S., Jónasson, J., Jonsson, P., Lundy, M., Martinelli, M., McAllister, G., Medvešek, D., Naseer, A., Reeve, C., Ulmestrand, M., Vacherot, J., Vila, J., A., Weetman, K., Wieland. 2020. Working Group on Nephrops Surveys (WGNEPS; outputs from 2019). 12-14 November, Split, Croatia. ICES Scientific Reports. 2:16. Annex 3: 79–83. ISSN number: 2618-1371 http://doi.org/10.17895/ices.pub.5968

The footage collected during the Adriatic UWTV surveys was usually analysed later in the institute laboratory by a team composed by Italian and Croatian scientists (Martinelli et al., 2017). Not all the footage collected during the UWTV survey can be considered as readable, hence some filters were already settled for the Adriatic footage in order to consider a station as a valid one: the achievement of a minimum of 7 minutes for each station, the level of turbidity lower than 3 NTU and a sledge speed that does not exceed 1.3885 knots and is not lower than 0.6592 (Martinelli et al., 2017; ICES, 2020). Furthermore, the detection of *Nephrops* burrows can be challenging and affected by subjective evaluations especially when occurring together with burrows of other species (e.g. Calocaris macandreae; ICES, 2017a). Hence, before starting each reading session, all the readers went through a training (or re-training) process aimed at familiarising with the characteristics of the footage. As suggested by ICES (2008, 2017a), a reference set to validate counter performance was created; a reference set for a specific area consists in footage of five minutes duration from different stations, covering different ranges of visibility (poor, medium, and good), varying in *Nephrops* density (low, medium, and high), and species aggregation. The final result per minute (reference count) is therefore the average count of 2 or more concordant readers. To create counts for this footage the expert readers reviewed it in isolation; results were compared and where significant differences between the counters occurred, the footage for that minute was re-examined until a consensus among the readers was reached. During the training sessions, the new readers must note the number of *Nephrops* burrows counted per minute in the reference footage. To measure the ability of counters ICES adopted the Lin’s coefficient of concordance (Lin, 1989). Given the nature of the UWTV footage (water clarity, variety of burrowing species present, etc.), the threshold for accepting an individual count should be ≥ 0.5, as recommended by the SGNEPS (ICES, 2009a). To be in line with ICES protocols a review of the historical Adriatic data series (2012-2017) was conducted evaluating the homogeneity of the readings through Lin’s CCC (ICES, 2020). The readings of 2009 and 2010 were excluded from the analysis because only consensus reading was used on all stations, therefore the validation process through Lin's CCC was not applicable. After application of speed and turbidity thresholds (Martinelli et al. 2016; Martinelli et al. 2017; ICES 2017b), all stations collected from 2012 to 2017 with a minimum of 7 readable minutes and evaluation from at least two readers (169 out of 250 stations) were tested for validation. A preliminary investigation on the results of the application of Lin's CCC method on the dataset showed that 27.8% of stations failed the Lin's CCC criteria (i.e. comparisons between couples of readers achieving a Lin's CCC value under the threshold of 0.5). In practice, a total of 47 stations out of 169 failed the test and therefore should be reviewed by additional counters. (Figure 3.3).

To explore a possible alternative, the Kendall correlation test was also trialled on the same dataset (Akoglu, 2018). The Kendall rank correlation coefficient (as the more common Spearman coefficient) is used to measure the ordinal association between two measured quantities with a statistical tau test (it is a non-parametric hypothesis test for statistical dependence based on a coefficient). A quantitative comparison between the number of stations validated by means of the Kendall coefficient and those by Lin's CCC was undertaken. The results showed a quite greater conservative nature for the Kendall coefficient compared to Lin's CCC, thus it was decided to continue using Lin's method as the concordance coefficient between readers (Table 3.1).

Table 3.1 The number of valid stations by year using Kendall coefficient and Lin's CCC. A station is considered valid when Lin's CCC > 0,5 for at least one couple of readers (or $\tau > 0{,}05$ for Kendall).

YEAR	**n° valid stations with Kendall ($\tau > 0.05$)**	**n° valid stations with Lin's CCC**
2012	7	14
2013	8	12
2014	12	28
2015	15	21
2016	27	32
2017	11	15

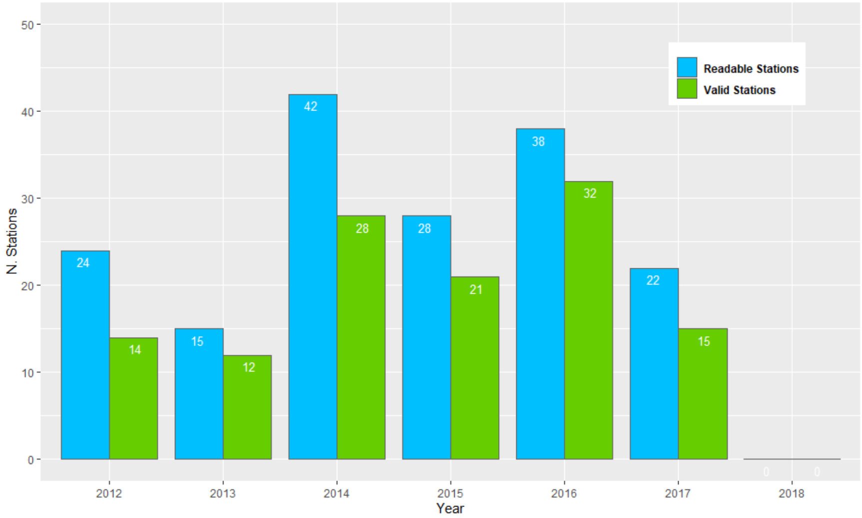

Figure 3.3 Yearly number of readable stations (blue) against number of validated stations (green) using Lin’s CCC.

Therefore, during the latest reading session held in Ancona from the 9th to the 20th of September 2019, the Lin’s CCC was applied for the first time to carry out: i) training on Adriatic Reference set and test on readers’ performances, ii) 2019 footage analysis and validation of stations during the readings, iii) revision of the 2012-2017 time series.

With the overarching goal to utilise as many stations as possible, in case of non-valid stations (Lin’s CCC < 0.5) new readings were performed in order to generate more couples to be tested via Lin’s CCC. If this strategy could not be applied, for instance in 2019 footage when no other trained readers were available during the session, the first and last minutes were removed before running a new Lin’s CCC because It’s supposed that these minutes could be the most affected by inattention, thus influencing counts precision.

During the readings of 2019, the above described protocol and the station validation method by means of Lin’s CCC were adopted, obtaining the validation of 70% of the total stations (Figure 3.4).

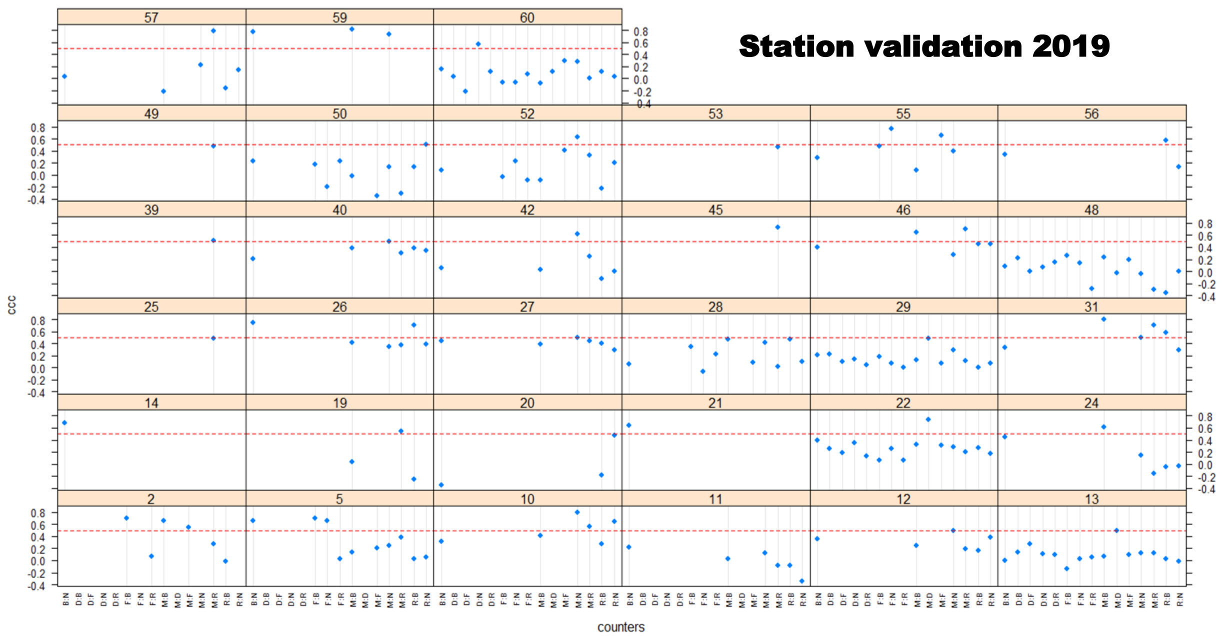

Figure 3.4 Results of 2019 Adriatic UWTV readings. On the y-axis the Lin's CCC and on the x-axis each pair of counters (indicate with letters). Each panel represents a station and the red line the recommended threshold of 0.5. To be considered as valid a station needs at least one couple of readers above 0.5 (included).

Furthermore, the same trained readers carried out a review of the 47 stations which had not passed the 2012-2017 time series validation analysis, thus improving the total number of valid stations by 7%. To increase such a percentage a further analysis on the unvalidated stations was conducted by removing the first and last minute (or only the first when it was not possible to remove both due to the low number of available minutes) from all the stations that have at least 8 minutes increasing by 39% the percentage of saved stations. Figure 3.5 shows the number of validated stations at the end of the process.

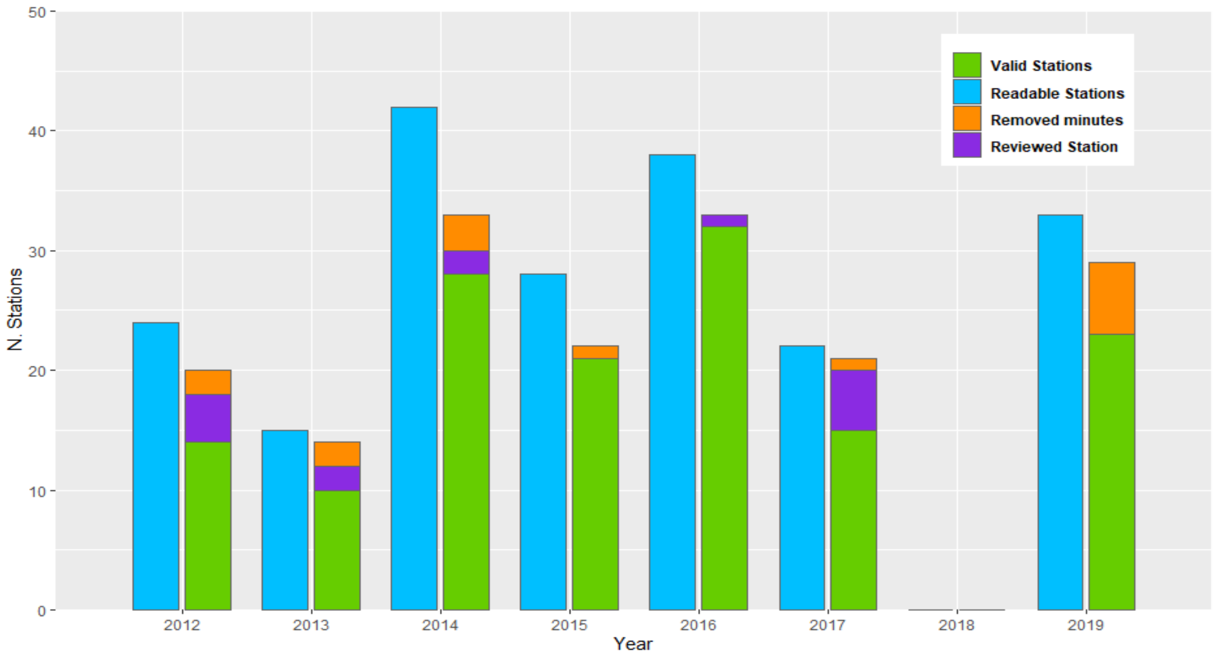

Figure 3.5 Number of readable stations against number of validated stations by year (in green stations validated after first Lin's CCC test run; in purple stations validated after addition of new readers, in orange station validated after removal of the first and last minute). A station was considered valid if Lin's CCC was above 0.5 for at least one couple of readers.

The obtained dataset was then integrated in the CNR database built by means of the Manifold® System Release 8 software (Martinelli *et al.*, 2017); the database allows setting of thresholds and application of biases along with the possibility to re-analysed all the time series according to new stratifications, such as the one based on the GFCM Pomo FRA zones. Before using historical series, several bias correction factors were thus applied in accordance with those adopted by ICES (Leocàdio et al., 2018): (i) 0.95 as detection bias related to the ability of the reader to detect *Nephrops* burrows actually present on the seabed, fixed for the area by experts; (ii) 1.25 as identification bias fixed by the area's experts on the basis of the presence of other burrowing megafauna and the ability to distinguish burrows made by different species; (iii) 1 as burrow occupancy assuming that one *Nephrops* occupies each burrow system counted (equal for all the areas); (iv) a variable bias *per* year and stratum (differently from the fixed bias adopted by ICES) as edge effect, excluding the number of burrow systems which partially lying out of the edge of the field of view (excluding approximately the 20% of the total number of counted burrows); (v) an additional surface bias adopted only for the Adriatic footage and accounting for the difference in length of the route covered by the sledge and the one covered by the vessel, based on the measurements made by means of a transponder newly installed on the sledge during the last survey (i.e. 2019). Figure 3.6 shows a comparison among the time series of average bias-corrected burrow densities obtained before and after the validation process.

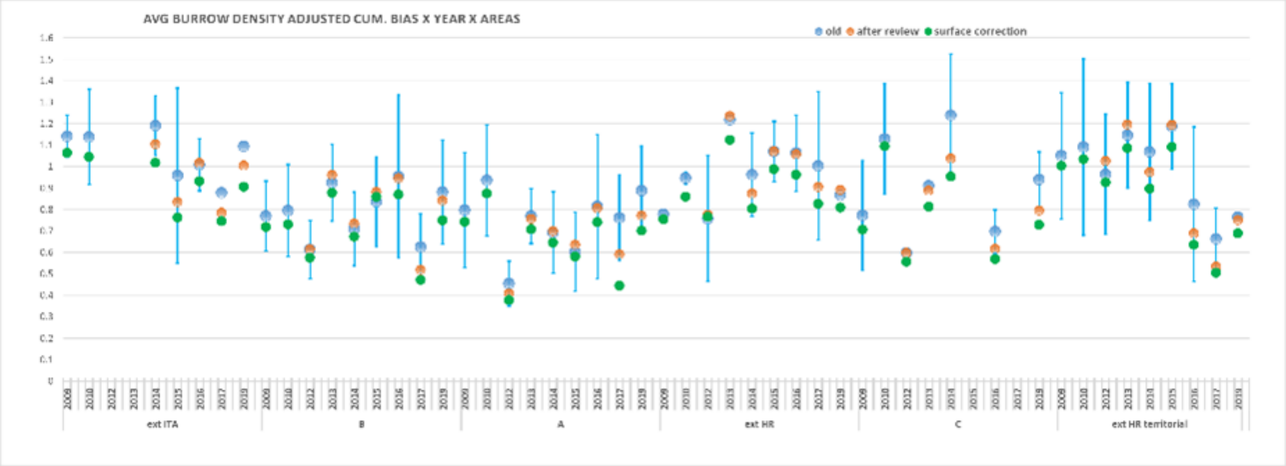

Figure 3.6. Average bias-corrected burrow densities (± standard error) by year and area (western to eastern) calculated before and after the validation process (blue and orange points respectively), and after the application of the surface correction factor (green points) for the entire UWTV time series (2009-2019). Years 2009 and 2010 were added for completeness of the historical series but have not been taken into consideration in the previous analyses.

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3.2 Burrow emergence rhythms of *Nephrops norvegicus* by UWTV and surveying biases

Abstract

Underwater Television (UWTV) surveys provide fishery-independent stock size estimations of the Norway lobster (*Nephrops norvegicus*), based directly on burrow counting using the survey assumption of "one animal = one burrow". However, stock size may be uncertain depending on true rates of burrow occupation. For the first time, 3055 video transects carried out in several Functional Units (FUs) around Ireland were used to investigate this uncertainty. This paper deals with the discrimination of burrow emergence and door-keeping diel behaviour in *Nephrops norvegicus,* which is one of the most commercially important fisheries in Europe. Comparisons of burrow densities with densities of visible animals engaged in door-keeping (i.e. animals waiting at the tunnel entrance) behaviour and animals in full emergence, were analysed at time windows of expected maximum population emergence. Timing of maximum emergence was determined using wave-form analysis and GAM modelling. The results showed an average level of 1 visible *Nephrops* individual per 10 burrow systems, depending on sampling time and depth. This calls into question the current burrow occupancy assumption which may not hold true in all FUs. This is discussed in relation to limitations of sampling methodologies and new autonomous robotic technological solutions for monitoring.

Keywords: Door-Keeping, Video-imaging, Burrow Emergence, Behavioural Rhythms, Catchability Patterns, Fishery-Independent Assessment, Optoacoustic.

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Contributions

All authors equally contributed to the analysis and writing of this work. J.D. and C.L. provided the WGNEPS field data from the UWTV surveys.

Introduction

The Norway lobster, *Nephrops norvegicus* (L.), is one of the most commercially important fisheries in Ireland and also Europe [1]. The 2019 EU Total Allowable Catch (TAC) for *Nephrops* [2] for the north east Atlantic Functional Units (FU) was close to 44,000 tonnes, and valued at approximately 360 million EUR in 2016 [3]. Traditional fishery-dependent sampling methods such as commercial trawling provide indirect biomass estimates of exploited stocks, by means of abundance indices derived from surface density data (i.e., the number of animals per haul-swept area [4,5,6]).

However, animals construct and inhabit burrow systems used for shelter and for territorial control [7] and are not available for trawl capture when hiding in the substrate [8,9]. The burrow emergence rhythmicity of populations causes marked fluctuations in catch rates over the 24-h [10]. Peaks in trawl Catch Per Unit Effort (CPUE) shift in timing with increasing fishing depth [11,12,13]: from full night to dusk- dawn transitions, going from upper to middle-lower shelves, to be finally fully diurnal (i.e. at midday) on upper and middle slopes. This indicates that the species sets its timing of burrow emergence upon a maximum illumination threshold that varies on the depth axis, based on the differential penetration of light as the sun progresses through its diurnal trajectory [10,14].

The diel rhythm of burrow emergence is more complex than previously thought and it can be subdivided in three different phases [11,15]: Full emergence, full retraction and an intermediate period in which individuals wait at the burrow entrance (i.e. door-keeping [16]). To date, the proportion of animals not emerging from their burrows on a daily basis is still largely undetermined, although acoustic tagging of individuals of a philetically closely related species has offered some insight [17]. In addition to environmental light, other ecological reasons seem to modulate the predisposition of individuals toward emergence or retraction. For example, crustaceans are at intermediate levels of the marine food webs and their feeding activity (coinciding with burrow emergence in the case of *Nephrops*) is the product of a mortality risk ratio between hunger state and chances to meet visual predators [18,19].

Alternative fishery-independent assessment methods as Underwater Television (UWTV) surveys using towed camera-sledges, have been developed to estimate stock abundance [20,21]. Those video-based surveys are carried out in several European countries and are coordinated through the International Council for the Exploitation of the Sea Working Group on *Nephrops* Surveys (WGNEPS) [1,22,23]. This more direct (i.e. image-based) method of assessment counts burrow systems, based on their characteristic structural features (i.e. large crater-like entrances [4,24,25] and their characteristic arrangement individual burrow entrances [20]. Those systems are composed of multiple entrances, shafts and tunnels and can be readily identified by classic features and orientation of the individual burrow entrances, where the apexes of those entrances facing each other in a simple U-shaped system or converging on one central point in a more developed system (i.e. T-shape) [26,27]. This method is independent from the time of the day and season. The burrow system counts can be used as a relative or absolute index for determination of *Nephrops'* stock status and together with catch data can provide a Harvest Rate (HR; catch in numbers/burrow numbers) [28,29].

To use UWTV burrow abundance to calculate catch and landings derived from an acceptable Harvest Rate (HR = catch in numbers/UWTV abundance) it is necessary to adjust using agreed correction factor which takes into account the bias associated with UWTV surveys. The key bias contributions for individual *Nephrops* Functional Unit (FU) have been documented and the cumulative correction factor considers edge effect, burrow identification, burrow detection and burrow occupancy [28,29].

Given the strong territorial behaviour of the species, burrow counting seems to be a good proxy for local population abundance, assuming the condition “one burrow system, one animal” [30], which is the current assumption for *Nephrops* stock assessment [28,29]. The burrow system acts as the center of a strong territorial rhythmic behaviour [7,31,32] and two adult lobsters are rarely found in the same shelter [33]. No spatial segregation occurs between juveniles and adults [34] and the majority of juveniles build adult-juvenile burrow complexes, which become separated as juveniles grow, and each individual develops its own section [35]. However, burrows systems could also be inhabited by other benthic fish and crustacean species or may remain empty and intact for an unknown period of time after the animals' death (e.g. due to fishing or natural mortality [30,36]). These factors still pose uncertainties about the true numbers of animals occupying video-counted burrow systems, representing a problem when using UWTV data in stock assessment models [30].

To improve knowledge of the stock assessment assumption “one burrow system, one animal” a precise temporal description of burrow emergence rhythmicity could be provided by temporally distributed transects within UWTV surveys, similar to that explored with trawl data [11]. The sum of animals engaged in both behaviours could then be compared with counted burrows at phases of maximum population emergence. Unfortunately, temporally scheduled UWTV operations have never been systematically performed and the analysis of rhythmic fluctuations in video-observed animals performing full emergence and door-keeping is not yet available.

Here, we used UWTV survey data reporting densities of full emergence and door-keeping animals and burrow systems from more than three thousand video transects, conducted in the past decade around Ireland, to temporally define both behaviours and their reciprocal relationship over the 24-h. Results on estimated densities of animals engaged in full emergence and door-keeping were then compared with burrow system density estimates, to provide a comparison to the stock assessment assumption “1 burrow system, one animal”.

Materials and Methods

The study areas and the UWTV surveys methodology

Video footage and derived data were collected from 3055 UWTV transects conducted from 2002 to 2013 in FU areas in the seas around Ireland (Fig. 1). Footage from each transect for all survey areas had a minimum recorded duration of 10 min. The counted minutes of each transect was in line with the prevailing international counting procedure; in years 2002 to 2008 10 min were counted, and in years 2008 to 2013 7 min were counted [28,29]. For FU 16, 10 min were counted for all years, due to the lower densities observed and the relative scale of variation between minutes was higher than typically found in other areas. All considered data were collected in spring–summer surveys (from May to September) (Table 1), in order to avoid variations in the number of video-counted animals and based on reproductive and moulting cycles (see next section).

Sampling design followed either a randomised isometric grid with a station spacing dependent on the individual survey area or a random stratified design [37] (Table 1). The initial ground perimeter was established by using a combination of integrated logbook-VMS [38] and habitat data (see methods described in Ligas et al. [39]). The final perimeter has been established using an adaptive approach where stations were located beyond the previously known perimeter of the ground, until the burrow system densities were zero or very close to zero. Once established, the survey area was not changed between years.

At each station, the UWTV sledge was deployed and once stable on the seabed, a 10 min’ tow was recorded onto DVD. The field of view of the camera (Kongsberg OE14-366) at the bottom of the screen with the sledge flat on the seabed (i.e. no sinking), was validated at 75 cm by two parallel spot lasers. Vessel position (by Differential Global Positioning System-DGPS) and sledge position (using an on-board Ultra Short Baseline-USBL, transponder) were recorded every 2 to 5 s. USBL navigational data were used to calculate the video transect distance over the ground, as required for surface density estimates (see next section). The navigational data were quality controlled using an “R” script developed by the Marine Institute [28,29].

Data processing

The same footage was viewed and counted by two scientists independently of each other and burrows were identified based on key structural features from an established set of classification keys [20,25,40]. All scientists were trained prior to counting following the ICES recommendations [28,29], in such a way the counters can be quite consistent in the recognition of a *N. norvegicus* burrow as compared to other species. Final burrow densities were based on an average from the two independent counts after passing quality control processes such as screening for outliers and use of Lin's concordance CCC to evaluate counter performance [20]. The quality assured burrow density values were then corrected for stock specific survey cumulative bias as described in ICES [29].

Video-counts of door-keeping animals, defined as those with partial cephalothorax or claws were visible across the burrow mouth entrance, and animals in full emergence which were entirely visible were available [10,15]. As with burrow systems, densities of animals in door-keeping and full emergence were obtained for each transect by dividing respective counts by the video-swept surface.

Each density estimate for burrow systems and animals in both behavioural categories was associated to a time stamp, represented by the time at mid transect length. All density data were grouped per depth ranges within the upper and lower shelf, based on the previous knowledge from trawl catch patterns [11,41,42] nominally as: 15–50, 51–100, 101–160 and 340–570 m. No data were available for 161–339 m depth. Data for the bathymetric range 340–570 m were only available in FU 16 for the years 2012 and 2013, and this inclusion was necessary to characterize behavioural rhythms in the deepest range for comparison with shallow shelf observations (as previously done with trawling [11]).

The rational for those depth groupings was that *Nephrops* burrow emergence is an adaptive life trait under strong selection which can be temporally described as different on upper and lower shelves as well as slopes [11]. Moreover, the burrow emergence rhythm manifests itself similarly in all its geographic range, with coincident nocturnal, crepuscular or diurnal timings according to the depth light driven peaks not blurred by the tidal status [10,43]. This behaviour is constant through years, subjected only to a seasonal reproduction and growth pattern (e.g. berried females do not emerge [15,44,45]), the effects of which were eliminated here by selecting only summer data (see Table 1).

Statistical analysis

Firstly, we ran a waveform analysis to describe averaged full emergence and door-keeping behavioural rhythms over the 24-h within the established depth ranges (see above). Waveforms plots describe the phase of a rhythm as an averaged peak into a time series of density data for both behavioural categories. Waveform computing procedure was as follows [46]. A standard day was divided into 1-h intervals and all density estimates for animals in full emergence and door-keeping were pooled together from all the surveys within the same depth range and then averaged at corresponding 1-h timing.

The resulting set of averaged density estimates were then represented over the 24-h with their standard deviations, plus the Midline Estimated Statistic of a Rhythm (MESOR [47]). MESOR is a re-average of all waveform values to be represented onto waveform plots as a horizontal threshold line. All waveform values above the threshold delimit the duration of the peak (i.e. activity peak duration [46]). Waveforms for full emergence and door-keeping density estimates were plotted together to highlight their temporal relationship.

Then, we fitted Generalised Additive Models (GAM) onto full emergence and door-keeping data for spring–summer at the established depth ranges (see above), to achieve statistic formalization of observed emergence patterns beyond data variability (Appendix 1). The package ‘mgcv’ [48] in R [49] was used with the restricted maximum log-likelihood approach. The effects of the inter-annual variability and the variability among FUs were assessed in the models. The Hour of the Day (HD), from zero to 23 h, was the covariate used to characterize behavioural rhythms. The day-length and the average location of the transects (latitude and longitude) were adopted as spatiotemporal covariates in the full model, following the form:

$$E(NEP)=g^{(-1)}\,(\beta 0+year+ FU+ s(HD,bs=cc,k=24)+s(Daylength)+ te(Lat,Lon)) \quad (1)$$

where *E(NEP)* is the Expected value of *Nephrops* full emergence or door-keeping, conditionally distributed according to the Gamma distribution family. *g* is the log link function, β0 is the intercept. *s* is the smoothing function with the term *bs* = "cc" specifying the 24 h’ knot based (k = 24) cyclic cubic regression spline. The day-length was estimated as the difference between the sunrise and sunset times. *te* is the tensor smooth function for the interaction among transect locations (i.e. latitude and longitude) accounting for spatial dependence on diel activity rhythms affecting *NEP*. Alternatively to the *te(Lat, Lon)* effect, the potential effect of the station locations per FU, *te(Lat, Lon, by = FU),* and the interaction between the station locations with the year survey *ti(Lat, Lon, year, d = c(2,1)*, were also tested in the models. The *ti* tensor product spline tested the significance of the space-year interaction effect. The 2-dimensional space and the 1-dimensional year factor were specified with the argument *d* = c(2,1).

The models showing significant HD term (i.e. behavioural rhythm), and other significant covariates substantially improving the model variance were selected as the final models (see Appendix 1). The different models were fitted and compared using the percentage of explained deviance and the Akaike Information Criterion (AIC) to select the best one [50]. The range of AIC values of models within depth ranges was generally narrow and not assumed to be critical for the final model choice. The selected final model followed the form (see Appendix 1):

$$E(NEP)=g^{(-1)}\ (\beta 0+s(HD,bs=cc,k=24)+f(Cov)\ ) \quad (2)$$

where *f*(*Cov*) represented the term *te(Lat, Lon)* for the emergence and door keeping behaviours in the upper depth range (15–50 m). In the case of the emergence behaviour at the depth ranges between 51 and 160 m and door keeping at 101-160 m, *f*(*Cov*) represented the term *s(Daylength)*. Because of the indistinguishable effect of the terms *te(Lat, Lon)* or *s(Daylength)*, on the *NEP* behavioural pattern (see Appendix 1), here we show results and focus on the behaviour produced by the HD term of *NEP*.

We averaged over periods of 1-h, and this probably resulted in models with relatively less variable behaviour during the day, even if the general distribution of *Nephrops* in full emergence and door-keeping did not show appreciable changes. The depth range models allowed identifying peaks timing and duration of full emergence and door-keeping behaviours from the fitted values above the mean.

Finally, in order to identify the temporally optimum moment to count the highest number of individuals (i.e. those in full emergence plus those in door-keeping) in relation to burrow counting, hence obtaining a best estimate of burrow occupancy, a temporally integrated chart of all waveform and GAM results phases [48,49] was created. Peaks were represented together as continuous horizontal lines and plotted in order to achieve an overall perspective of their temporal relationship [51,52].

Results

Waveform analysis (Fig. 2) indicated that *Nephrops* full emergence varied from nocturnal toward midday hours with increasing depth of sampling. This pattern is particularly evident, when comparing the two extremes of the sampling depth range: upper shelf (15–50 m depth) with two peaks (hour intervals: 2 to 9 and 18 to 0) *versus* middle slope (340–570 m depth) with single peak (hour interval: 9 to 17). At intermediate sampling depths of the lower shelf (51–100 m depth) and shelf-break (101–160 m depth), a less clear crepuscular (dusk and dawn oriented) pattern was reported, with less distinct peaks merging toward daytime. In contrast, door-keeping behaviour had some defined pattern with crepuscular peaks coinciding with full emergence only on the upper shelf (15–50 m depth) and the shelf-break (101–160 m depth). No defined rhythms were discernible at the other depth zones.

The statistical model results by GAM (Fig. 3), revealed an overall pattern of full emergence and door-keeping behaviour similar to that found from the waveform analysis (Fig. 2). In the upper continental shelf (15–50 m depth), the model shows a nocturnal bimodal (i.e. two peaks) emergence pattern (hour intervals: 2 to 7 and 17 to 23). On deeper shelf areas (from 51 to 160 m depth), the emergence pattern becomes diurnal with a plateau shape (i.e. no major crepuscular peaks). Finally, in the upper slope (340–570 m depth) the emergence shows a single peak (hour interval: 7 to 17).

Consistent with the waveform analysis (see Fig. 2), door-keeping, showed a less clear temporal pattern (Fig. 3). As with emergence, door-keeping was nocturnal with weak crepuscular increases at 15–50 m depth range (hour intervals: 0–7 and 15–0). The temporal pattern was lost between 51 and 100 m to be regained with a crepuscular aspect on the shelf break (hour intervals: 4–7 and 15–21), becoming again completely arrhythmic on the upper slope.

The temporal comparison between the waveform analysis (see Fig. 2) and GAM model outputs (see Fig. 3) in peak timings and duration per depth stratum is presented in Fig. 4. In the upper shelf (i.e. 15–50 depth), GAM modelling indicated a slightly shorter timing of nocturnal emergence. At intermediate and lower shelf (from 51 to 160 m depth), the waveform and GAM analysis shows a slight drop of emergence at noon. On the slope (340–570 m depth), the midday timing for emergence indicated for waveform analysis (hour interval: 9–17) was modelled by GAM as taking place for a longer duration (hour interval: 7–17).

The same comparison for door-keeping behaviour (see Fig. 4) showed a nocturnal rhythmicity at depths 15–50 m with both waveform analysis and GAM, with a duration slightly larger for the latter. Although no significant temporal pattern was detected by the GAM modelling from 51 to 100 m depth and from 340 to 570 m depth, on the shelf-break some weak crepuscular temporization was detected by the two analysis approaches.

Independently of the survey time, the maximum densities of emergence and door-keeping were detected in the 51–100 m depth layer (means of 0.058 and 0.020 Ind./m^2, respectively, in the FU 15, West Irish Sea), coinciding with the maximum number of burrows per area (0.908 burrows/m^2) (Table 2). This corresponds to a visible occupancy of 0.086 individuals per burrow.

The timing of the maximum number of animals in full emergence varied between depth strata (Fig. 4, Table 3). The mean densities of animals ranged from 0.024 and 0.061 Ind./m^2 over the continental shelf (from 15 to 160 m depth), and one order of magnitude lower on the slope (340–570 m depth) with densities between 0.0064 and 0.009 Ind./m^2.

Focussing on mean density values for all visible animals (combined totals of both emergence and door-keeping behaviours) at peak timing as a proxy of total population densities (Fig. 4), the following observation can be made: the animal density increases from 0,034 to 0,075 Ind./m^2 over the continental shelf, and from 0,012 to 0,013 Ind./m^2 over the slope. The fraction of door-keeping animals (i.e. from the total emergence and door-keeping animals) is slightly lower on the continental shelf than on the slope (18–41% and 32–54%, respectively) (Table 3). The number of total animals visible per burrow system ranged from 0,059 to 0,119 Ind./m^2 across the shelf and the slope.

Discussion

The present work describes for the first time the diel behavioural rhythms of *Nephrops* in terms of burrow emergence and door-keeping, based on observations in more than three thousand

UWTV transects. Populations emergence patterns varied their timing from the shelf to the slope with a timing shift, which is consistent with previous observations based on trawl catch temporal rates (i.e. in capture peaks from nocturnal to crepuscular and then to fully diurnal hours as the depth increases [11]). In contrast, the description of the temporal variation in door-keeping behaviour is an entirely new finding for *Nephrops*, since individuals at the entrance of their tunnel systems are unlikely to be catchable in trawling operations [53]. Here, we provide evidence of arrhythmic fluctuations in counts of animals expressing this behaviour, with relevant counts sparse over the whole 24-h cycle. This points out that the arrhythmia of observations of door-keeping animals could be due to: a behaviour which is in fact arrhythmic in some individual, or that animals retract into the burrows because they perceive approaching sleds. An unknown part of the population may therefore avoid haul capture by a quick withdrawal of individuals into their burrows when trawls approach [54]. Consequently, the number of counted "door-keeping animals" could be dependent upon sledge towing speed (i.e. animals reacting to the approach of the sledge and retreating [25]), as well as the presence of the sledge light system and overall noise. In any case, for trawl gear to efficiently catch Norway lobsters, they have to be fully outside of the burrows.

Burrow emergence rhythms and the UWTV-based stock assessment assumptions

Estimated densities of visible animals engaged in both emergence and door-keeping behaviours were compared with burrow system counts and derived density estimates, to provide evidence putative biases to the standard stock assessment assumption that "1 burrow system is occupied and maintained by one animal" [20]. Present results suggest a visible individuals' ratio of around 1 visible *Nephrops* to 10 counted burrows. This result is a general estimate considering all the FUs, with results closer to the 1:1 assumption in some areas (i.e. FU 15). Taken together, our results indicate that there may well be variations in the burrow occupancy across different FUs. In fact, this difference appears to actually be related to the latitude/longitude position rather than the FU as the GAM showed FU to be irrelevant, while sample location (or day length) was influential in the model (Appendix 1). Video-derived animal densities during the consecutive hours were variable and the minimum estimates of stock densities should be derived focusing on the hours at which maximum densities are visible at the surface. Temporal windows at which we reported maximum emergence (plus door-keeping) densities of *Nephrops* can be considered good time periods to compare animals and burrow systems numbers together.

This burrow occupancy assumption has been identified a major uncertainty in UWTV bases assessment approach, particularly when using the UWTV index as an absolute measure of stock abundance [20]. Field observations indicate a more complex behavioural situation where single individuals can inhabit a single or complex burrow system with a variable number of entrances depending on local population densities [30]. At the same time, laboratory studies on aggressive hierarchy show that dominant individuals attempt to evict the subordinates to conquer their burrows nearby [9]. Even in periods of peak emergence it is possible that not all individuals are visible at the surface. Aguzzi et al. [18] suggested that the predisposition of animals toward burrow emergence depends on the hunger state (and the presence of carrion and prey) and the absence of potential predators or sympatric competitors. Furthermore, laboratory tests on large numbers of individuals indicate that shelf animals may exhibit a differently phased dusk or dawn emergence, possibly to reduce interspecific competing pressure [13]. This matches present results where the temporal patterns in emergence observed in *Nephrops* UWTV transects generally follow those described by trawl catches on the shelf. The environmental factors such as the lunar cycle, tides and bottom currents, whose strength could vary according to the different local topography in different FUs, could also impact on burrow emergence [16]. Depending upon the future spatiotemporal availability of environmental data (to date missing) new variables could also be modelled, to improve the model correction approach.

It is possible that the number of burrow systems are over estimated, for a variety of reasons. Despite the training systems in place to ensure consistency in *Nephrops* burrow identification, the accuracy of burrow identification may vary across FUs. In some areas sympatric fish and other decapod species occupy or even construct burrows with morphology similar to those of *Nephrops* [30]. It is also possible, in environmentally stable lightly trawled grounds, that unoccupied burrows may persist, and appear to be active (clearly inactive systems are not counted). However, most of the grounds in our study are heavily trawled with swept area ratios > 5 [28,29]. The number of burrow entrances per counted burrow system may also be variable in different habitat types. It is unlikely that all these factors can fully account for the discrepancy in the animals to burrow count ratios observed here between areas.

Considering our results and the previously accounted sources of uncertainty for the UWTV-based stock assessment equation, our estimation of the general value of “1 Ind./10 burrow correction factor” does call into question the use of UWTV surveys as an absolute index on *Nephrops* abundance. A visible *Nephrops* index does provide a minimum population estimate of those emerging on a diel basis, but may not account for those concealed. The Irish Sea (FU 15) is a very dynamic system with strong bottom currents and highly populated sea bed [28,29] so the 1:1 assumption could likely hold in that area. At the same time, this assumption could be quite different in FU 20–21, which is less fished and has lower densities of burrow systems. The HR for FU 15 has for long periods been around 20% and that observation clearly invalidates any possibility that our ratio of 0.1 Ind./burrow can be close to the true value (since the local fishery would be catching twice the number of animals/burrows annually). Still that doesn’t mean that the ratio 1:1 is true for all other FUs.

Trawling is a traditional sampling approach for the scientific monitoring of demersal resources but it does not provide data on the behaviour of the target species nor how such a behaviour can influence catchability [51]. UWTV surveying has distinct advantages over trawling, being more ecologically sensitive, causing minimal physical damage to seabed habitats and allowing better behavioural characterization. However, considerable work remains to be done in order test the key assumptions used in the assessments based on UWTV survey programmes.

The methodological constrains of our study based on input data typology

We chose to group the data for depth ranges and across years rather than keeping at a higher level of granularity, since *Nephrops* behaviour is usually highly variable [55]. In fact, the fitted GAMs suggested that there are no significant effects of the survey year (i.e. inter-annual variability) on the emergence or door keeping patterns. The models also suggested that the variability across FUs is not relevant (i.e. significant) in explaining the behavioural patterns.

Additional data sub-grouping based on light data (not available) could have been performed. However, the estimation of any environmental illumination index based on transect timing, geographic position and depth would result in a mere modelling exercise. Factors such as cloud cover, water column primary productivity and turbidity have a significant impact on light scattering and absorbance (i.e. extinction) coefficients [56,57], and are unavailable at the high spatiotemporal frequency of UWTV transects for all the FU areas considered.

Moreover, *Nephrops* rhythmicity is part of burrowing behavioural life-styles under natural selection in crustacean decapods, which was shown to be expressed independently from contingent variations in background light intensity [58]. The species possesses a biological clock that would ensure a temporally averaged burrow emergence pattern. The biological clock activates the locomotor activity (at the base of burrow emergence), every 12-h or 24-h, depending from the shelf or slope depth stratum considered [13,59]. This rhythmicity is self-sustained since it keeps its period and phase based on an environmental memory of previously experienced environmental light conditions when animals are transferred to laboratory constant conditions (i.e. entrainment upon intensity and photophase duration) [47,60].

Another source of data variation may be the underlying dynamics of the populations due to recruitment variations, fishing and natural mortality [39]. In the case of the Mediterranean *Nephrops* stocks, fishery overexploitation is not reducing the number of captured animals but the biomass (i.e. animals are getting smaller) [61]. To date, there is no evidence of a similar finding for the Irish Sea. The local stocks are not experiencing declines due to excessive fishing mortality (e.g. FU 15, has continuously yielded ~ 10,000 tonnes of catch for nearly 60 years) [62]. It is feasible that a behavioural mechanism modulating emergence is preserving the populations from the fishery exploitation (see all considerations above).

Toward a more technologically sustained fishery-independent stock assessment

Towing the UWTV sledge could bias counts of emerged individuals causing them to flee outside the field of view and cause door-keepers to retract inside their burrows [53]. To improve stock assessments, more intensive data collection efforts are needed to collect data for improved models. Data collection may include optoacoustic by multi-beam cameras that should be used in combination with High-Density (HD) imaging from Autonomous Underwater Vehicles (AUVs) [63] and Internet Operated Vehicles (IOVs) such as crawlers [64]. A complete photomosaic of the targeted parcel describing burrow systems and their reciprocal positioning, should be undertaken as a first step. Only after that, hourly scheduled AUV and IOV' acoustic sweeps should be continuously performed during consecutive day-night cycles, replicated in different seasons, to picture emerging and door-keeping *Nephrops* and associated predator and prey species under silent and non-light conditions.

In addition, different stand-alone or cabled observatories, holding several seabed and water column sensors for environmental monitoring (e.g. the OBSEA or SmartBay; respectively, https://www.obsea.es and https://www.smartbay.ie/), could be used to picture burrow emergence modulation (for an insight on monitoring network geometry and characteristics see [65,66]). The different observational points could be synchronously used to account for the control of oceanographic and ecological drivers on the burrowing behaviour of the species [67]. With such a multidisciplinary demographic, behavioural and environmental approach one may finally derive more accurate stock assessment models, predicting the density of animals that could be sampled with different fishery-dependent and independent tools [67].

Conclusions

Our results highlight that *Nephrops* is highly cryptic and has fascinating behavioural patterns that affect its availability to visual as well as capture-based surveys. The temporal treatment of UWTV video data within the chosen depth ranges showed the behavioural pattern of burrow emergence is predominantly dusk and dawn-oriented above 50 m, bimodal and tending to be diurnal between 50 and 100 m, temporally diffused between 101 and 160 m, and finally fully diurnal between 340 and 570 m, partially matching depth-dependent patterns in trawl catch rates. The door-keeping behaviour is only temporally defined above 50 m (being nocturnal) and bimodal with a nocturnal increase between 100 and 160 m. During the hours of maximum peak abundance of visible individuals (summing up the video-counted individuals in emergence and door-keeping behaviours), we have observed that on average there is about 1 visible individual per 10 burrows, at most. This represents the average peak, although there were higher peaks within individual transects. In general, considering all areas together, our ratio is well below that assumed in current stock assessments (i.e. "1 burrow system:1 animal"), suggesting that a high proportion of the population remains cryptic even during periods of peak emergence. This bias should be carefully considered since an undefined number of animals may avoid the sledge at its approach. Further technological development toward optoacoustic technologies and additional effort for calibration and modelling to integrate observations of visible individuals may further improve the utility of UWTV surveys for stock assessment. Four lines for technological based calibration should be foreseen in future stocks monitoring actions: burrow identification, as other sympatric fish and decapod species occupy or even construct burrows with morphology similar to those of *Nephrops*; intraspecific aggressive relationships and hierarchy, where dominant individuals may occupy different burrow systems nearby; emergence enhancement and inhibition depending on hunger state, due to predators' presence and the quantity of emerging conspecifics at the "rush hour timing"; and finally burrow persistence after an animal's death, depending on the density of burrow-dwelling species, local hydrographic and fishing pressure conditions.

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Table 1. FU grid spacing is shown for randomised isometric sampling designs. Random stratified sampling is used for FU19. The distribution of UWTV surveys across summer months and number of transects in each FU is also shown.

FU Code	Area	Depth range (m)		Sampling months					Number of UWTV transects
		Min	Max	May	Jun	Jul	Aug	Sep	
15	West Irish Sea	15	162				▨	▨	1501
16	Porcupine Banks	343	570		▨		▨	▨	115
17	Aran grounds, Galway Bay, Slyne Head	26	162	▨	▨	▨	▨		854
19	South and SW coasts of Ireland	18	116		▨	▨	▨	▨	101
20-21	Labadie, Jones and Cockburn Banks	95	138			▨	▨	▨	125
22	The Smalls	74	145		▨	▨			635

Table 2. Time-independent density of animals (Ind./m^2) at emergence and door-keeping per depth range by *Nephrops* functional units (FUs).

FU Code	Depth range (m)	Number of UWTV transects	Emergence (ind/m^2)		Door keeping (ind/m^2)		Burrow density (bur/m^2)	
			Mean	SD	Mean	SD	Mean	SD
15	15-50	349	0,022	0,054	0,008	0,016	0,622	0,606
	51-100	754	0,058	0,087	0,020	0,025	0,908	0,517
	101-160	398	0,042	0,076	0,017	0,021	0,895	0,458
16	340-570	115	0,003	0,006	0,003	0,006	0,124	0,061
17	15-50	71	0,016	0,031	0,015	0,019	0,849	0,394
	51-100	261	0,015	0,035	0,009	0,017	0,393	0,374
	101-160	522	0,019	0,038	0,015	0,019	0,678	0,354
19	15-50	5	0,000	0,000	0,006	0,010	0,396	0,321
	51-100	55	0,005	0,014	0,003	0,007	0,292	0,276
	101-160	41	0,005	0,012	0,004	0,007	0,379	0,258
20-21	51-100	4	0,010	0,015	0,002	0,004	0,187	0,374
	101-160	121	0,003	0,016	0,003	0,007	0,387	0,287
22	51-100	267	0,009	0,023	0,005	0,010	0,261	0,236
	101-160	368	0,019	0,037	0,008	0,013	0,571	0,331

Table 3. Mean density (Ind./m^2) of *N. norvegicus* and burrow occupancy at periods with the predicted highest (maximum) number of animals displaying full emergence and door-keeping behaviour, as identified in Fig. 4.

Depth range (m)	**Period (hour)**	**Emergence (Ind/m^2)**	**Door keeping (Ind/m^2)**	**Emergence + door keeping (Ind/m^2)**	**Fraction of emergence %**	**Fraction of door keeping %**	**Burrows (bur/m^2)**	**Burrow occupancy (Ind/bur)**
15-50	03 -04	0,024	0,011	0,034	69,2	30,8	0,579	0,06
	04 -05	0,057	0,013	0,070	81,6	18,4	0,734	0,10
	19 - 20	0,037	0,010	0,046	79,0	21,0	0,655	0,07
	20 - 21	0,043	0,012	0,055	78,1	21,9	0,645	0,09
51-100	07-08	0,045	0,015	0,060	74,9	25,1	0,600	0,10
	08-09	0,061	0,015	0,075	80,2	19,8	0,736	0,10
	15-16	0,054	0,015	0,069	77,7	22,3	0,582	0,12
101-160	05-06	0,034	0,010	0,044	77,5	22,5	0,517	0,08
	06-07	0,041	0,017	0,058	71,1	28,9	0,654	0,09
	18-19	0,027	0,015	0,042	63,1	36,9	0,652	0,06
	19-20	0,026	0,018	0,045	59,0	41,0	0,722	0,06
340-570	11-12	0,006	0,007	0,012	45,7	54,3	0,162	0,08
	12-13	0,009	0,004	0,013	68,3	31,7	0,130	0,10

Figure Legends

Figure 1. The UWTV survey areas around the Irish coast. Numbers represent the ICES *Nephrops* Functional Units (FUs), as defined by the ICES *Nephrops* Working Group. Depth (m) for the study areas (GEBCO bathymetry data). Map created using: R version 3.61 (2019–07-05) software, https://tinyurl.com/yy6rzlut.

Figure 2. Waveform analysis results depicting the change in burrow emergence behaviour upon depth in terms of full emergence (black) and door-keeping (red). MESORs are the threshold horizontal dashed lines (respective values are also reported with corresponding colour), which identify peak temporal limits (i.e. values above it; coloured vertical arrows). The peak duration is an indication of global averaged activity for that behavioural component in the population. Separate peaks were identified if 2 or more consecutive points were below the MESOR..

Figure 3. Significant ($p < 0.05$) GAM modeled temporal patterns for full emergence (grey) and door-keeping (red) behaviours by depth ranges over the 24-h cycle. Shadowed areas represent 95% confidence intervals of modelled patterns. Horizontal black dashed lines are the zero-mean values, taken as a reference to estimate representative time ranges of full emergence and door-keeping activity peaks (i.e. values above the mean). Door-keeping model fits at 51–100 and 340–570 m depth ranges were not significant ($p > 0.05$).

Figure 4. Integrated chart showing temporal relationships among peaks timings, as derived from plots of waveform and GAM analyses (i.e. phases as values above horizontal threshold lines; see Figs. 2 and 3). According to GAMs, the best timings of UWTV surveying where highest (maximum) number of animals can be observed, are indicated with the letter M and grouped by blue rectangles.

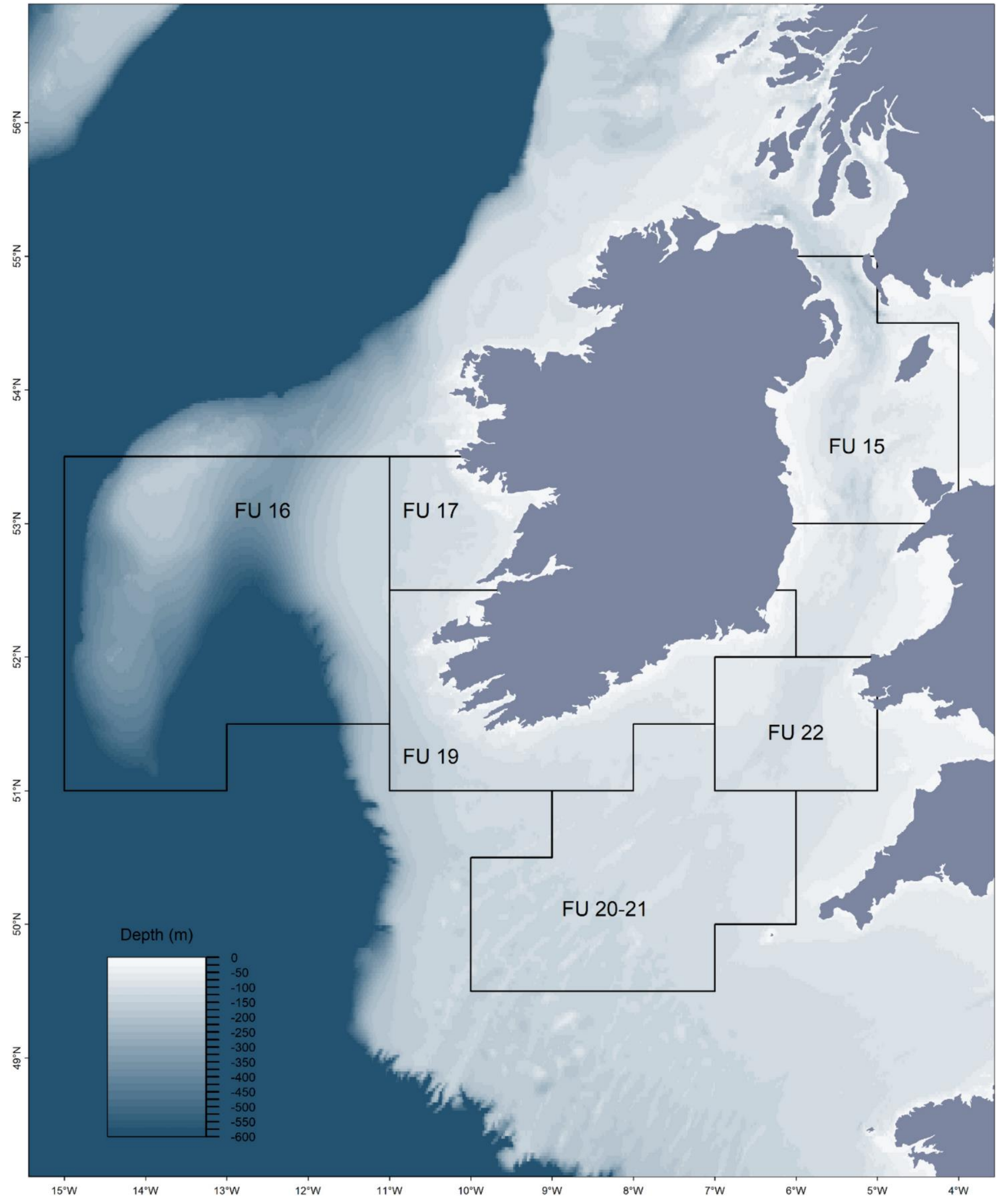
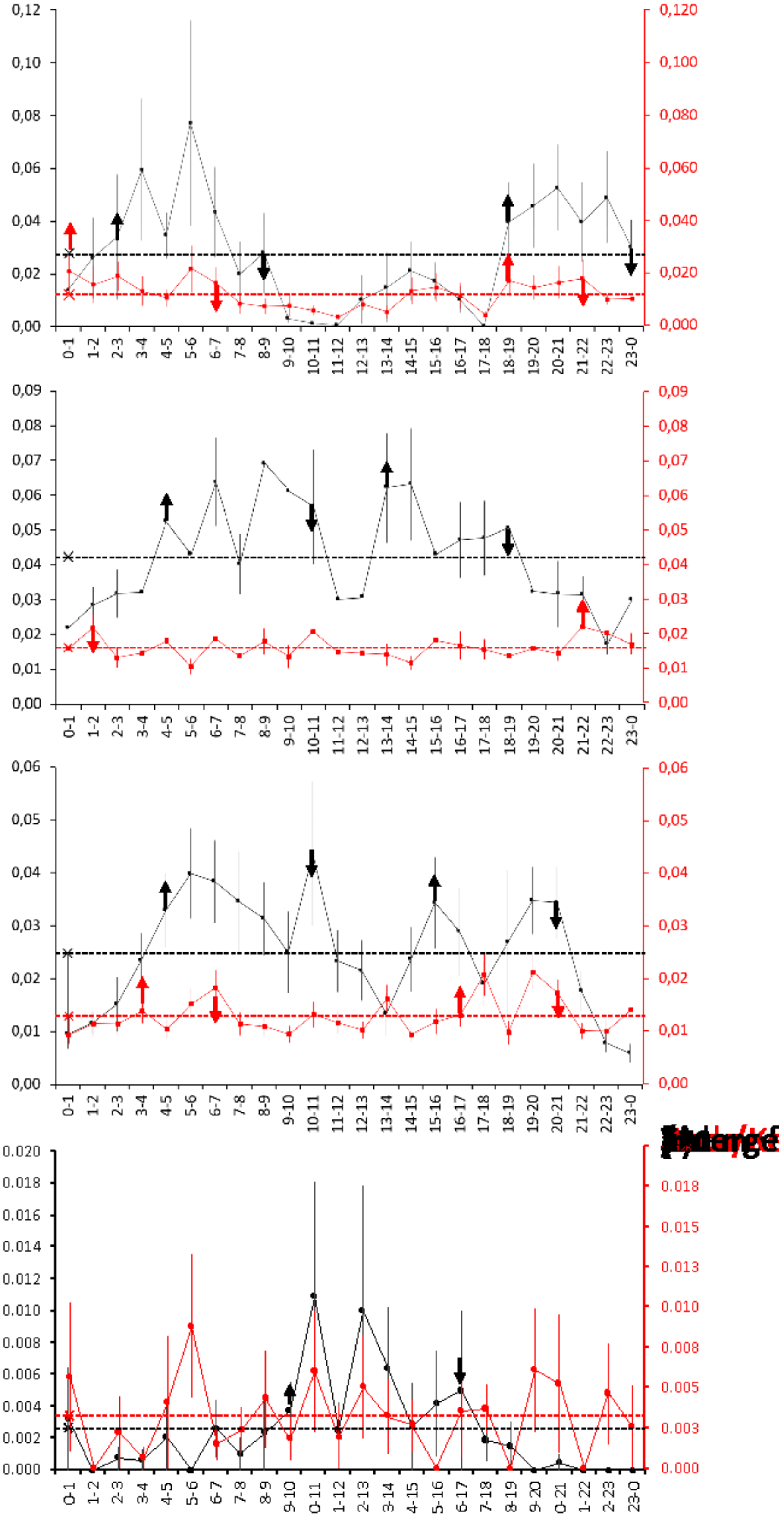

FU 15
FU 16
FU 17
FU 19
FU 20-21
FU 22
Depth (m)
0
-50
-100
-150
-200
-250
-300
-350
-400
-450
-500
-550
-600
56°N
55°N
54°N
53°N
52°N
51°N
50°N
49°N
15°W
14°W
13°W
12°W
11°W
10°W
9°W
8°W
7°W
6°W
5°W
4°W

Fig. 1


0,12
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9-10
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11-12
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13-14
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21-22
22-23
23-0
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1-2
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23-0
0.020
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0.000
0.018
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0.008
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2-3
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10-11
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18-19
19-20
20-21
21-22
22-23
23-0

Fig. 2

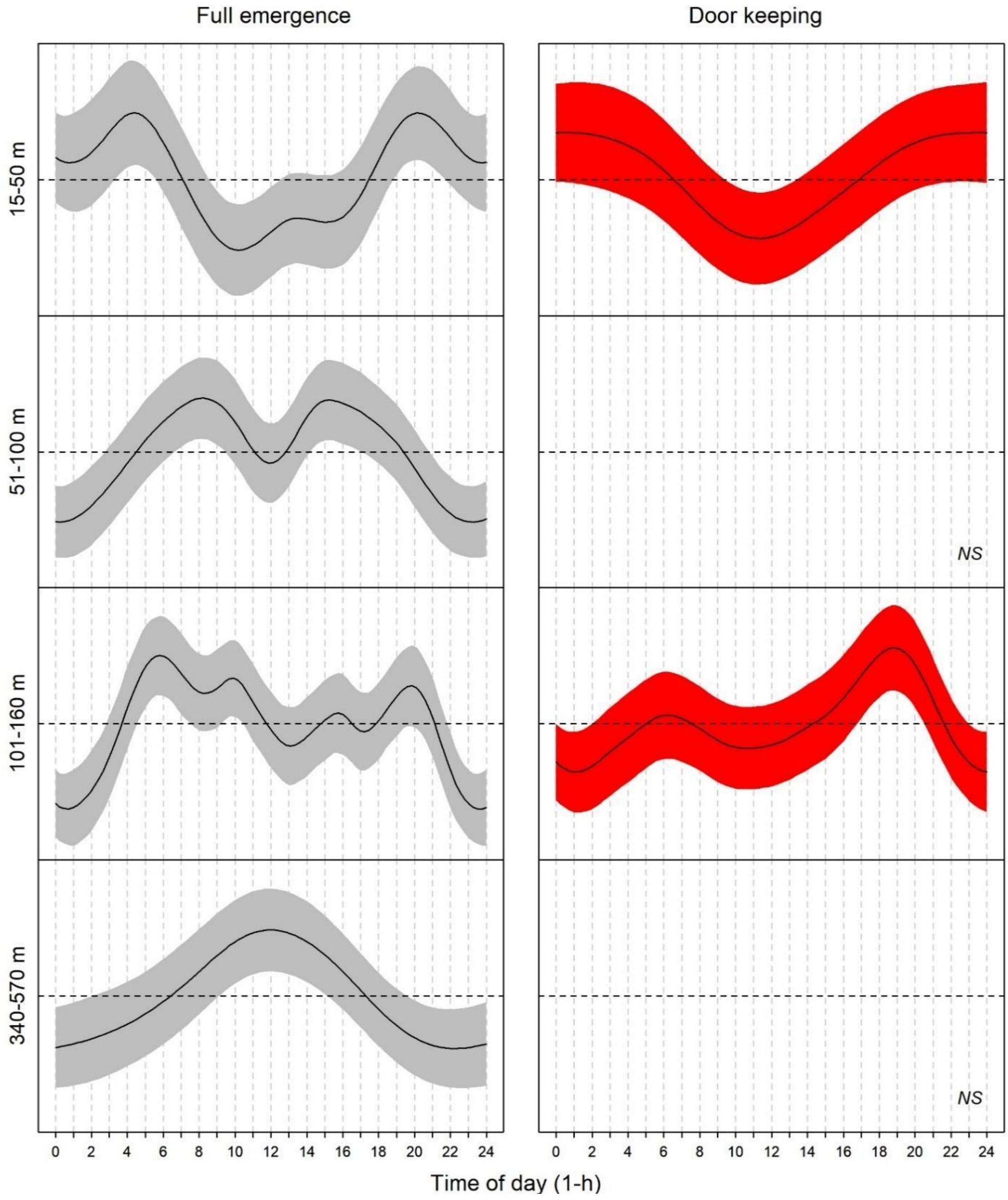
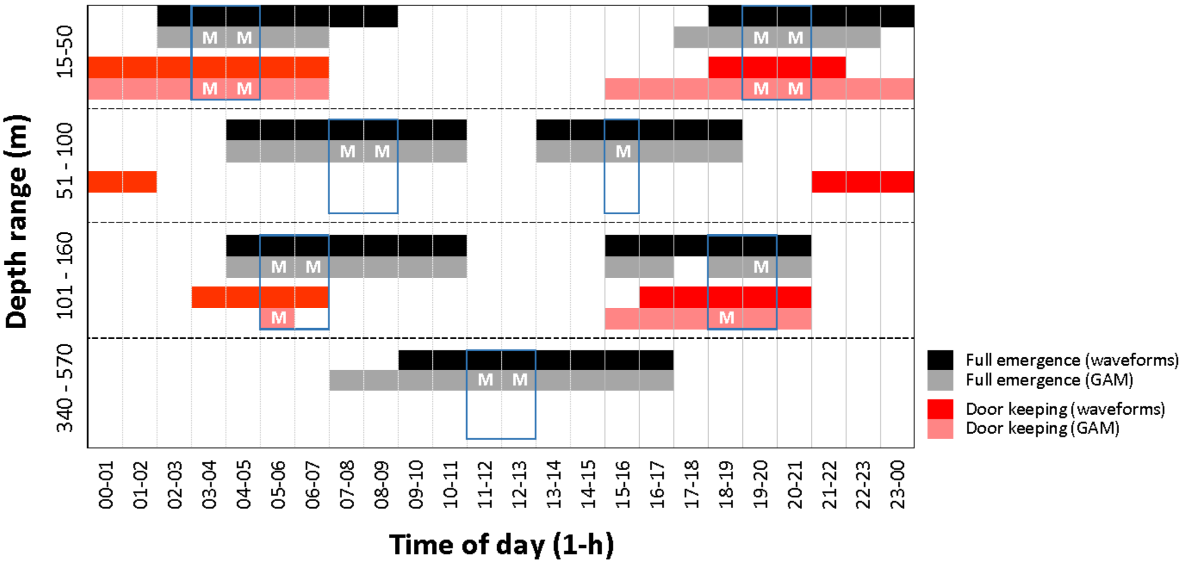

Full emergence
Door keeping
15-50 m
51-100 m
101-160 m
340-570 m
NS
NS
0
2
4
6
8
10
12
14
16
18
20
22
24
Time of day (1-h)

Fig. 3



Depth range (m)
15-50
51 - 100
101 - 160
340 - 570
M
00-01
01-02
02-03
03-04
04-05
05-06
06-07
07-08
08-09
09-10
10-11
11-12
12-13
13-14
14-15
15-16
16-17
17-18
18-19
19-20
20-21
21-22
22-23
23-00
Time of day (1-h)
Full emergence (waveforms)
Full emergence (GAM)
Door keeping (waveforms)
Door keeping (GAM)

Fig. 4

Appendix 1

Stepwise selection of the best-fitting GAMs Models (Mod). A number of GAMs were fitted to investigate for the effect of the year survey, Functional Units (FUs), day-length and transect coordinates on the *Nephrops* (NEP) behaviour. The models were adjusted separately for each stablished depth range, from 15 to 570 m depth for surveys from data between 2002 and 2013 (**Table A1**). Final models were selected (with covariates in bold; **Table 1A** and **Figure 1A**) based on the significance of the HD term and other significant covariates strongly improving the total model variance. The range of AIC values was relatively narrow within depth ranges and it was assumed not to be critical for model choice. Thus, models were disregarded if showing no significant covariates or showing significant covariates such as year and FU, but not strongly improving the model variance (e.g. Mod 1 to Mod 4 for the emergence behaviour with the depth range 51-100 m). Apart from the significant effect of the Hour of the Day (HD), the contribution of transect locations and day-length to explain the chosen models variability is indistinct (e.g. Mod 10 for emergence behaviour at depth ranges between 51-100 and 101-160, **Figure 1A**). This is probably due to the fact that the correlations between day-length and latitude (*r* = 0.45) and longitude (*r* = 0.58) are relatively high. Note that, even though the correlation values are relatively high and may be redundant in the model, in some models they were tested together to assess for potential effects of the fraction of uncorrelated data. The final model choice only includes the transects location or the day-length (apart from HD); this suggests that the variability among years and the variability among FUs are irrelevant for explaining the behavioural pattern of *Nephrops*. Even the nested effect of the transect locations into the FUs and the interaction between the transect locations and the survey were not significant in the selected models.

Table A1. GAMs fitted to explain *Nephrops norvegicus* behaviour (emergence and door keeping) based on the survey year, Functional Units (FU), the day-length and station locations in the Atlantic Sea around Ireland. The *s* is the smoothing function for the hour of the day (HD) and day-length. *bs* = "*cc*" indicates the 24-h knot based (*k* = 24) cyclic cubic regression spline ("*cc*"). *te* is the tensor smoother for the spatial covariates (i.e. latitude and longitude). The *by* = FU argument nested the station locations into FUs. The *ti* tensor product spline tested the significance of space and time interaction. The argument *d* = *c*(2,1) indicates the function that the smooth consists of tensor product between space (2-dimensional smooth for longitude and latitude) and time (1-dimensional term, year). Models 4 to 8 were not fitted for the depth range 340-570 m, because of limited data (i.e. two years' data for a single FU). Selected models (shown in **Figure 1A**) are indicated with covariance values in bold.

Behaviour	Depth range (m)	Covariates	Mod 1	Mod 2	Mod 3	Mod 4	Mod 5	Mod 6	Mod 7	Mod 8	Mod 9	Mod 10	Mod 11	Mod 12
			p-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value
Emergence	15 - 50	Year (2002 to 2013)	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*					
		FU (15, 17, 19)	*NS*	*NS*	*NS*	*NS*					*NS*			
		s (HD, bs = "cc", k = 24)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		s (Daylength)	*NS*	< 0.001		< 0.001	*NS*	*NS*	0,012	NS	*NS*		*NS*	
		te (Lat, Lon)	< 0.001		< 0.001		< 0.001			< 0.001	< 0.001	< 0.001		
		te (Lat, Lon, *by* = FU)						*NS*^{e}						
		ti (Lat, Lon, Year, *d* = c (2,1))				*NS*			*NS*					
		Variance explained (%)	27,0	14,6	25,8	16,1	27,1	27,0	16,8	22,0	22,0	20,9	10,2	7,9
		AIC	-1349	-1349	-1345	-1314	-1350	-1345	-1316	-1345	-1343	-1343	-1311	-1309
	51 - 100	Year (2002 to 2013)	*NS*^{a}	*NS*^{f}	*NS*^{f}	*NS*^{a}	*NS*^{f}	*NS*	*NS*					
		FU (15, 17, 19, 20-21, 22)	< 0.001	< 0.001	< 0.001	< 0.001				< 0.001	0,032			
		s (HD, bs = "cc", k = 24)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0,020	< 0.001	< 0.001	< 0.001
		s (Daylength)	< 0.001	< 0.001		< 0.02	< 0.001	< 0.001	< 0.001	< 0.001	0,004		< 0.001	
		te (Lat, Lon)	< 0.001		< 0.001		< 0.001				0,003	< 0.001		
		te (Lat, Lon, *by* = FU)						*NS*^{e}						
		ti (Lat, Lon, Year, *d* = c (2,1))				*NS*			*NS*					
		Variance explained (%)	29,0	20,5	28,1	21,3	28,9	29,4	21,1	25,0	25,1	22,2	13,4	2,7
		AIC	-3251	-3152	-3239	-3151	-3248	-3248	-3141	-3193	-3196	-3167	-3066	-2947
	101 - 160	Year (2002 to 2013)	*NS*^{e}	*NS*^{e}	*NS*^{e}	*NS*	*NS*^{e}	*NS*^{e}	< 0.001					
		FU (15, 17, 19, 20-21, 22)	*NS*	*NS*	*NS*	*NS*					*NS*			
		s (HD, bs = "cc", k = 24)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		s (Daylength)	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	
		te (Lat, Lon)	< 0.001	< 0.001	< 0.001		< 0.001			< 0.001	< 0.001	< 0.001		
		te (Lat, Lon, *by* = FU)						*NS*						
		ti (Lat, Lon, Year, *d* = c (2,1))				< 0.001			< 0.001					
		Variance explained (%)	26,2	20,4	21,6	27,9	24,9	25,9	28,9	21,6	23,0	14,5	15,8	3,7
		AIC	-4737	-4680	-4683	-4716	-4732	-4740	-4723	-4694	-4702	-4598	-4630	-4464
	340 - 570	Year (2012, 2013)	*NS*	0,010	0,007									
		FU (16)												
		s (HD, bs = "cc", k = 24)	*0,001*	< 0.001	< 0.001						< 0.001	< 0.001	0,001	0,001
		s (Daylength)	*NS*	0,030							0,009		0,030	
		te (Lat, Lon)	*NS*		0,022						0,014	*NS*		
		te (Lat, Lon, *by* = FU)												
		ti (Lat, Lon, Year, *d* = c (2,1))												
		Variance explained (%)	33,2	28,8	24,1						34,0	20,6	15,6	11,8
		AIC	-869	-869	-866						-871	-862	-860	-857
Door keeping	15 - 50	Year (2002 to 2013)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001					
		FU (15, 17, 19)	*NS*	*NS*	*NS*	*NS*					*NS*			
		s (HD, bs = "cc", k = 24)	0,011	*NS*	0,009	*NS*	0,011	0,011	*NS*	0,021	0,017	0,014	NS	0,039
		s (Daylength)	*NS*	*NS*		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*		0,005	
		te (Lat, Lon)	< 0.001		< 0.001		< 0.001		*NS*	< 0.001	< 0.001	< 0.001		
		te (Lat, Lon, *by* = FU)						*NS*						
		ti (Lat, Lon, Year, *d* = c (2,1))				*NS*								
		Variance explained (%)	34,2	21,9	33,0	24,3	34,4	35,4	24,3	21,5	21,3	20,9	6,1	1,7
		AIC	-2222	-2175	-2222	-2178	-2225	-2224	-2182	-2179	-2176	-2179	-2138	-2127
	51 - 100	Year (2002 to 2013)	*NS*^{f}	*NS*^{f}	*NS*^{f}	*NS*	*NS*^{f}	*NS*^{f}	*NS*					
		FU (15, 17, 19, 20-21, 22)	< 0.001	*< 0.001*^{f}	< 0.001	*NS*^{f}					< 0.001			
		s (HD, bs = "cc", k = 24)	0,019	*NS*	0,019	*NS*	*NS*	< 0.001	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
		s (Daylength)	*NS*	*NS*		*NS*	*NS*	*NS*	< 0.001	< 0.001	< 0.001		< 0.001	
		te (Lat, Lon)	< 0.001		< 0.001		< 0.001			< 0.001	< 0.001	< 0.001		
		te (Lat, Lon, *by* = FU)						*NS*^{a}						
		ti (Lat, Lon, Year, *d* = c (2,1))				*NS*			< 0.001					
		Variance explained (%)	27,5	20,5	27,5	21,3	26,2	28,9	21,1	22,2	23,6	16,8	13,9	0,6
		AIC	-6266	-6195	-6268	-3151	-6248	-6274	-3141	-6195	-6211	-6124	-6115	-5950
	101 - 160	Year (2002 to 2013)	*NS*^{c}	*NS*^{c}	*N*^{f}	*NS*	*NS*^{c}	*NS*^{c}	*NS*					
		FU (15, 17, 19, 20-21, 22)	*NS*	*NS*	*NS*	*NS*^{f}					*NS*			
		s (HD, bs = "cc", k = 24)	0,001	0,002	0,003	< 0.001	< 0.001	0,001	0,002	< 0.001	0,002	< 0.001	0,004	0,025
		s (Daylength)	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	0,005	0,006		< 0.001	
		te (Lat, Lon)	0,022		0,013		< 0.001		< 0.001	< 0.001	*NS*	< 0.001		
		te (Lat, Lon, *by* = FU)						*NS*^{e}						
		ti (Lat, Lon, Year, *d* = c (2,1))				< 0.001								
		Variance explained (%)	17,3	14,8	15,1	25,4	18,3	17,6	23,8	13,5	12,2	11,7	8,4	1,0
		AIC	-7712	-7705	-7692	-7775	-7725	-7716	-7769	-7666	-7650	-7655	-7631	-7537
	340 - 570	Year (2012, 2013)	0,028	< 0.001	< 0.001									
		FU (16)												
		s (HD, bs = "cc", k = 24)	*NS*	*NS*	*NS*						*NS*	*NS*	*NS*	*NS*
		s (Daylength)	0,043	*NS*							< 0.001		< 0.001	
		te (Lat, Lon)	0,020		*NS*						< 0.001	0,017		
		te (Lat, Lon, *by* = FU)												
		ti (Lat, Lon, Year, *d* = c (2,1))												
		Variance explained (%)	33,6	27,1	30,5						30,7	10,8	29,7	< 0.1
		AIC	-880	-875	-877						-877	-853	-872	-846

[a] The interaction *te*(Lat, Lon): FU 15 was significantly different from the other interactions ($p<0.05$).

[b] The year 2003 was significantly different from the reference year 2012 ($p<0.05$).

[c] The years 2003 and 2005 were significantly different from the reference year 2012 ($p<0.05$).

[d] The years 2003 and 2004 were significantly different from the reference year 2012 ($p<0.05$).

[e] The FU 20-21 was not significantly different from the reference FU 15 ($p>0.05$).

[f] The FU 19 was significantly different from the reference FU 15 ($p = 0.009$).

[g] The interaction *te*(Lat, Lon): FU 15 and FU 17, were significantly different from the other interactions ($p<0.05$).

[h] The years 2005, 2006, 2008 and 2009 were significantly different from the reference year 2012 ($p<0.05$).

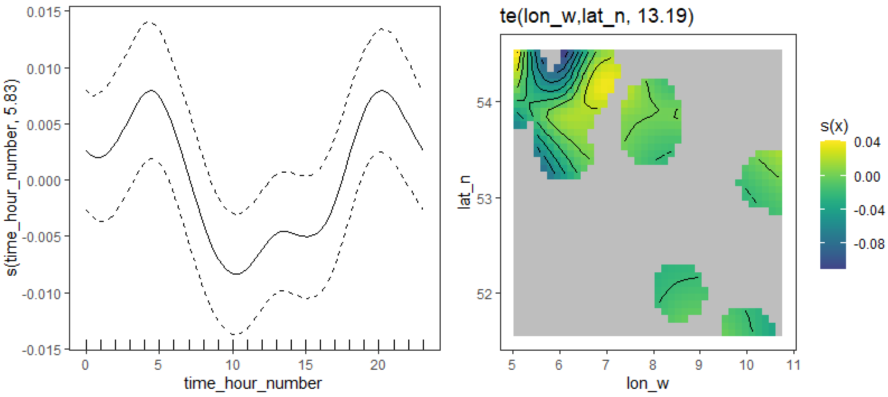
[I] The years 2006 and 2009 were significantly different from the reference year 2012 ($p<0.05$).

[j] The FU 22 was significantly different from the reference FU 15 ($p = 0.001$).

Figure A1. Effects of explanatory variables on the emergence and door-keeping behaviour at the different depth ranges, as estimated by the selected GAMs. The model (Mod) number and depth ranges are shown, as indicated in the **Table A1**. The plots were generated using the package 'mgcv'[48] in R[49].

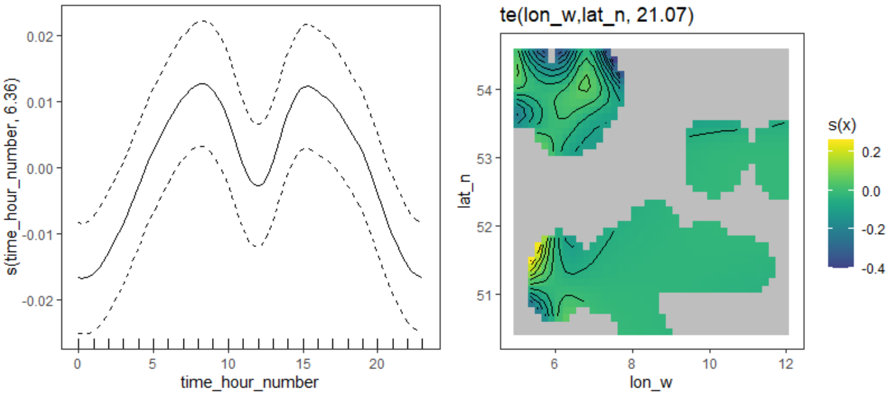
Emergence

Mod 10: $E(NEP) = g^{-1}(\beta_0 + s(HD, bs = cc, k = 24) + te(Lat, Lon)\,)$



15-50 m

Mod 10: $E(NEP) = g^{-1}(\beta_0 + s(HD, bs = cc, k = 24) + te(Lat, Lon)\,)$



51-100 m

Mod 11: $E(NEP) = g^{-1}(\beta_0 + s(HD, bs = cc, k = 24) + s(daylength)\,)$

51-100 m

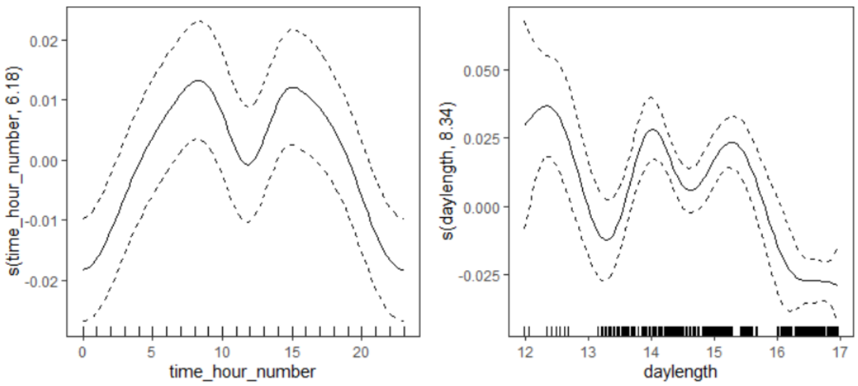
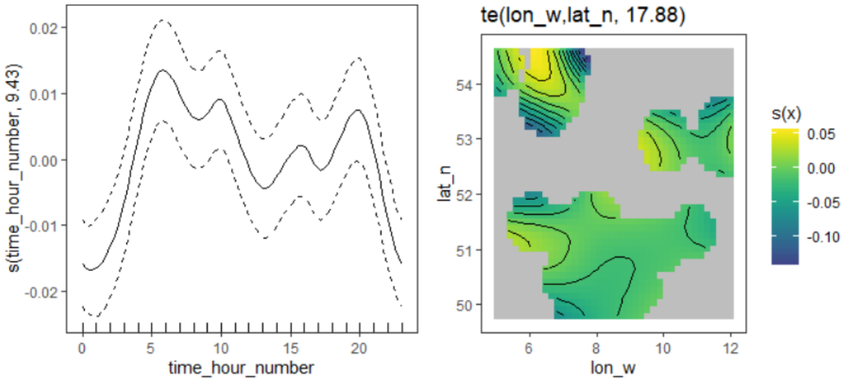

s(time_hour_number, 6.18)
0.02
0.01
0.00
-0.01
-0.02
0
5
10
15
20
time_hour_number
s(daylength, 8.34)
0.050
0.025
0.000
-0.025
12
13
14
15
16
17
daylength

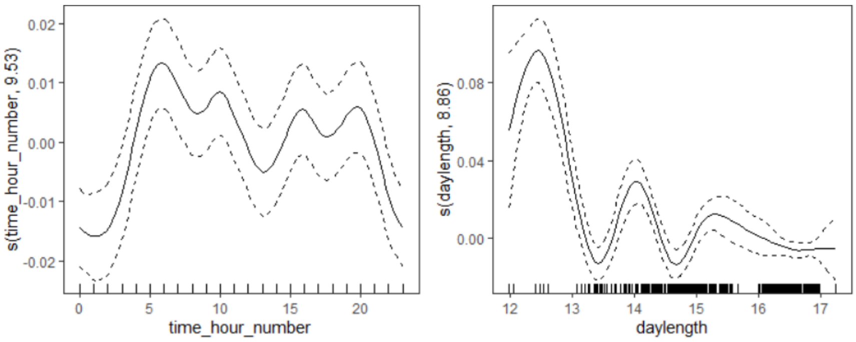
Figure A1. Cont.

Mod 10: $E(NEP) = g^{-1}(\beta_0 + s(HD, bs = cc, k = 24) + te(Lat, Lon))$



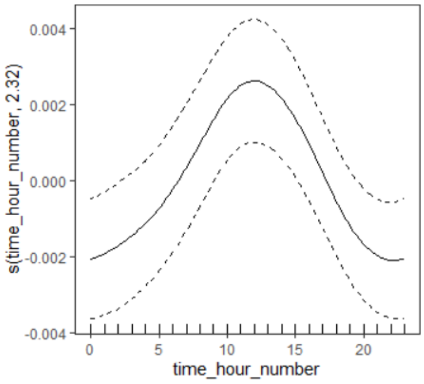
101-160 m

Mod 11: $E(NEP) = g^{-1}(\beta_0 + s(HD, bs = cc, k = 24) + s(Daylength))$



101-160 m

Mod 12: $E(NEP) = g^{-1}(\beta_0 + s(HD, bs = cc, k = 24))$

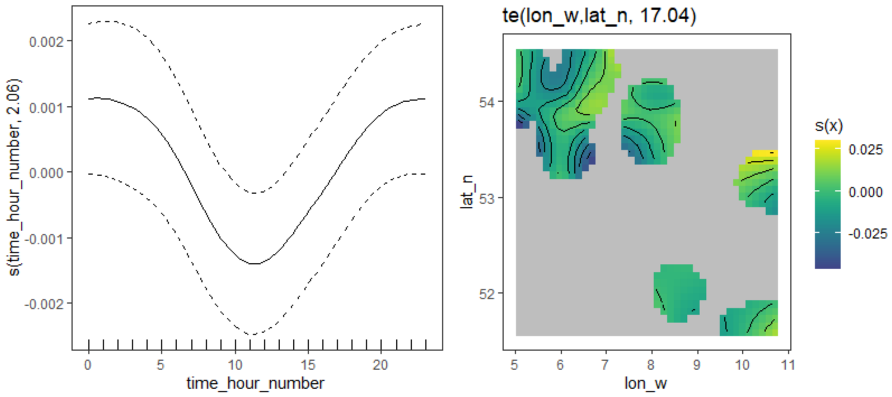


340-570 m

Figure A1. Cont.

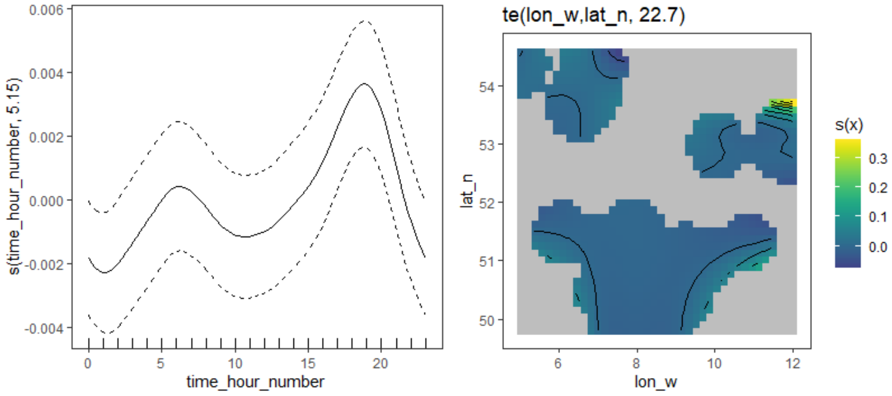
Door-keeping

Mod 10: $E(NEP) = g^{-1}(\beta_0 + s(HD, bs = cc, k = 24) + te(Lat, Lon))$



15-50

Mod 10: $E(NEP) = g^{-1}(\beta_0 + s(HD, bs = cc, k = 24) + te(Lat, Lon))$



101-160

4. *Nephrops* bottom trawl surveys

4.1 Looking for a simple assessment tool for a complex task: short-term evaluation of changes in fisheries management measures in the Pomo Pits area (Adriatic Sea)

Abstract

In this study, a BIAMS (Before–Intermediate–After Multiple Sites) analysis, namely a modified version of the BACI (Before–After–Control–Impact) approach, was used to evaluate the possible effects of the fishery management measures implemented in the Pomo/Jabuka Pits area (Central Adriatic Sea). The study area is characterized by a peculiar bathymetry and oceanographic regime, which make it an ideal habitat for *Nephrops norvegicus* and a nursery area for *Merluccius merluccius*; furthermore, ecologically interesting modifications in species assemblages and/or abundance rates have been observed in the area in the last decades. This is a historically highly exploited ground for Italian and Croatian fisheries, whose impact may have contributed over the years to modify the ecosystem. For this reason, since 2015, the Pomo/Jabuka Pits area was subject to various fishing regulations changing in type of restriction and over time and space, until the definitive establishment in 2018 of a Fishery Restricted Area (FRA); FRAs are usually considered useful tools to contribute to the recovery and maintenance of fish stocks and, as in this case, may include different levels of protection over space (i.e. no take zones and partially regulated zones). The evaluation of the effects of the fishery management measures implemented in the area was thus made complicated by these changes in regulatory regime over time and space, which may result in complex signals to be interpreted. The analysis was carried out on abundance indices (i.e. kg/km^2 and N/km^2) obtained in the period 2012-2019 for 5 commercially and/or ecologically important species, obtained by means of 2 annual trawl surveys carried out in the study area by the CNR-IRBIM of Ancona. The BIAMS approach was based on the selection of a *Closure* factor, declined in 3 levels (i.e. BEFORE/INTERMEDIATE/AFTER) and accounting for regulation changes in time, and of 3 adjacent strata (i.e. “A”, “B” and “ext ITA”) *a posteriori* determined according to the latest regulations. The temporal selected temporal dimension allowed the estimation of early effects of the measures on the analysed indices. The BIAMS was design to overcome the issue of the unavailability of a proper independent control site, in fact in strata “A” and “B” the levels of fishing effort varied over time according to the management measures in place, while in the last strata no restrictions have been applied to fishing activity; furthemore the selection of adjacent strata allowed the inference of possible interactions among them. The use of BIAMS allowed to identify a series of early effects (i.e. changes in abundances) through the implementation of different management fishing measures in time and space in a complex and very relevant area.

Keywords: BIAMS, Adriatic Sea, FRA, management measures

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1. INTRODUCTION

Industrial fisheries, in particular bottom trawling, is known to have important effects on marine ecosystems, potentially leading to alteration or degradation of habitats and overexploitation of marine resources [1]. Proper management is therefore crucial to ensure the sustainability over time of fishery resources and to protect the ecosystems [2,3]. Several management strategies can be adopted such as the control of fishing effort, the application of rules concerning fishing gears, the definition of the minimum landing size and closures in time and space [3].

It is well known that spatial closures to human activities (e.g. fishing, mining, dredging and dumping, etc..) meant for protection of sensitive marine ecosystems and/or vulnerable species [4–6] can also generate benefits for the productivity of commercially exploited fish stocks by protecting Essential Fish Habitats (EFH, i.e. areas in which fish spawning, breeding, feeding or growth to maturity occur; [7] and by promoting export of adults and larvae to adjacent areas [8–10].

Marine Protected Areas (MPAs) represent a classic and useful tool to protect marine biodiversity by limiting the effects of multiple human activities [6,11]. They may have various primary management goals (e.g. protect biodiversity, safeguarding ecosystem) and thus be implemented through different levels of regulation [12]. The consequent levels of protection established define various types of MPAs [6] which include for example no-take marine reserves (namely No Take Zones, NTZs), Partially Protected Areas (PPAs; [13]) or multiple-zone MPAs including areas subjected to different levels of protection [14]. Generally NTZs represent the extreme case of the precautionary approach banning all the potentially damaging activities (e.g. fishing, anchoring, SCUBA diving; [15], while PPAs aim to find a compromise between habitat preservation and human interests [16].

Fisheries Restricted Areas (FRAs) are instead geographically-defined areas in which restrictions on fishing effort and/or fishery ban have been implemented specifically in order to manage some important resource and/or protect EFHs with the final goal to contribute to the recovery and maintenance of fish stocks [11,17–20]. In FRAs there can be temporary and permanent closures in addition to regulations specifically targeting particular fishing gears [12].

The persistence over time of spatial management measures can modulate the achievement of biodiversity restoration or re-stocking of exploited populations [6]. In fact, while some benefits may occur quickly after protection measures implementation (i.e. short-term effect; [21–23]), others might take longer periods (e.g. decades) to manifest (i.e. long-term effect; [24,25]). Short term effects can occur within a few years, as described for example by Claudet et al. [22] for the Couronne and Carry MPAs (northwestern Mediterranean), where in few years since the establishment of the MPA, richness in species diversity and their abundances showed marked increments; however, in general to verify more stable effects on the ecosystem, long term observations are necessary. According to Claudet et al. [26] older European marine reserves are more effective than newly established ones in the restoration of commercial fish natural population age/size structures (especially for long-lived species). Furthermore, long term effects may also include increments in rare and vulnerable species, and recovery of degraded habitats [27]. To evaluate the possible effects over time of spatial management measures, different approaches can be adopted: identification of possible changes in the ecosystem composition, variations in the biological parameters of certain species such as growth or fecundity rate, or abundance indices of species of particular importance for the area under protection [12,28]. The choice between one or more of these indicators depends on the primary objectives of protection and the pre-existing knowledge of the characteristics of the area and the ecosystem in general [29]. For example, if the specific goal of a marine spatial closure is the recovery of target populations of commercial fish, indicators as density and size, which are supposed to be affected by fisheries closures, should be used [30].

Before–After–Control–Impact (BACI) analysis [31] used to be considered a rigorous design for assessing the impact of MPAs [13]. The general BACI design involves the measurement of an ecological variable of interest (i.e. ecological indicators), that is expected to be positively or negatively affected by a management action, before and after the implementation or a change in the management strategy in a specific site and, as a comparison, in one or more not impacted control sites [31]. Unfortunately, scientists are not often in the position to adopt that ideal design (e.g. when the protected area is already established, or when sampling approaches are limited and do not allow temporal and/or spatial replication) [32]. Claudet and Guidetti [33] stated the need for adapting sampling and analytical designs to the temporal and spatial framework of a protected area with the overarching goal to improve the assessments of the management measures adopted. Historically, several alternatives to the general BACI design were used to evaluate the effectiveness of spatial closures, in cases where the ideal design was not applicable [34–41], for example adopting deconstructed sampling designs with replicated controls as After-Control-Impact (ACI) designs, with temporal and spatial comparisons after the establishment of the spatial closure [42], or Control-

Impact (CI) designs accounting only for spatial comparisons [43]. If a spatial control is lacking, the effects of spatial closures should be inferred only by sampling Before and After an impact (BA design; [34]). Actually, control sites are ideally unaffected by spatial management measures, thus they should be ecologically and physically similar, have the same type of species assemblages and similar habitat features compared to the potentially impacted area but must also be statistically independent [36,40]. Practically, the sea is spatially and temporally dynamic, and finding two locations that are statistically identical, subject to the same environmental conditions and independent one to one another is often problematic, resulting in being one of the major limitations for the application of the BACI evaluation approach [44]. Also in this case alternatives to the classic BACI design were provided [44,45]. For example, Methratta et al. [46] suggested a Before-After-Gradient (BAG) design which entirely eliminates the need to identify suitable and valid control by sampling multiple sites along a spatial gradient within and around a wind farm (or in and around a marine reserve; [47]. Another limitation of the classic BACI design could be the binary temporal dimensions (i.e. before vs. after), which could mask potential changes occurring on a finer time scale induced for example by environmental variability over time (e.g. climate change) or implementation of simultaneous management approaches [34,36]. For example, periodic examination of data, when compared to before–after, could reveal the time at which an AMP starts to be effective [44].

The Mediterranean basin offers plenty of cases for which the effectiveness of spatial management measures has been rarely demonstrated mainly due to the lack of the basic requirements to develop appropriate sampling designs and/or assessment [12,48]. Among the Mediterranean ecoregions, the Adriatic Sea is recognised as a priority area for conservation purposes [49]; the reason behind this need for protection is the high fishing pressure which over time has caused the degradation of marine habitats, decline of target and non-target species, food-web alterations and loss of biodiversity [50–53]. In fact, the Northern-Central part of the Adriatic Sea represents the European area most intensively fished by bottom trawlers [54].

In particular, the central part of the Adriatic Sea, characterised by three depressions delimited by the 200 metres bathymetry (having a maximum depth of about 270 metres; [55]), together known as Pomo (or Jabuka in Croatian) Pits, is one of the main fishing grounds within this basin, shared by the Italian and the Croatian fleets [56,57]. According to Russo et al. [57] the main fishing zone for the Italian fleet targeting Norway lobster and hake is the one located just south of the Pomo Pits (Fig. 1). The complex topography of the area, combined with the oceanographic regimes of the Adriatic Sea, makes this a very peculiar environment, in which the water exchange does not occur every year [58]. These conditions can influence the nutrient cycle, with consequences on local biodiversity and on the trophic status of benthic communities [59]. This area is the main nursery for european hake, *Merluccius merluccius* (Linnaeus, 1758), in this basin [60–63]. Furthermore, the presence of muddy bottoms and other exogenous factors make it an ideal habitat for Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758) [64]. Numerous studies reported that here the population of Norway lobster is characterised by high densities of individuals smaller, and growing slower, than those from other areas of the Adriatic [65–69]. Among the other crustacean species occurring in the area, a commercial and ecological relevance is attributable to the pink shrimp, *Parapenaeus longirostris* (Lucas, 1846), that in the last decade showed a relevant abundance increase in the Mediterranean Sea [70,71]. An abundance peak occurred in the Pomo/Jabuka Pits in 2017; furthermore, as described by Martinelli et al. [72], this species shows periodic fluctuations in the area, which could also be linked to environmental parameters changes (e.g. salinity and temperature; [73]). A crustacean species shift also occurred in the Pomo/Jabuka Pits area: *Munida intermedia* (Milne-Edwards & Bouvier, 1899) was in fact almost completely replaced by *Munida speciosa* (von Martens, 1878), first observed in 2003 [74]. This species replacement was also observed in other areas of the Mediterranean and linked to climate change and to intrinsic characteristics of the species [75]. Alteration in species assemblages and possible consequences on trophic and ecosystemic balances could probably be due to the synergistic action of the fishing pressure and climate change [74,76,77]. Also another gadoid species dwelling in the area, the blue whiting *Micromesistius poutassou* (Risso 1827), was proven to experience fluctuations in abundance over time as a result of environmental and hydrodynamics variations mixed to fishing exploitation [78].

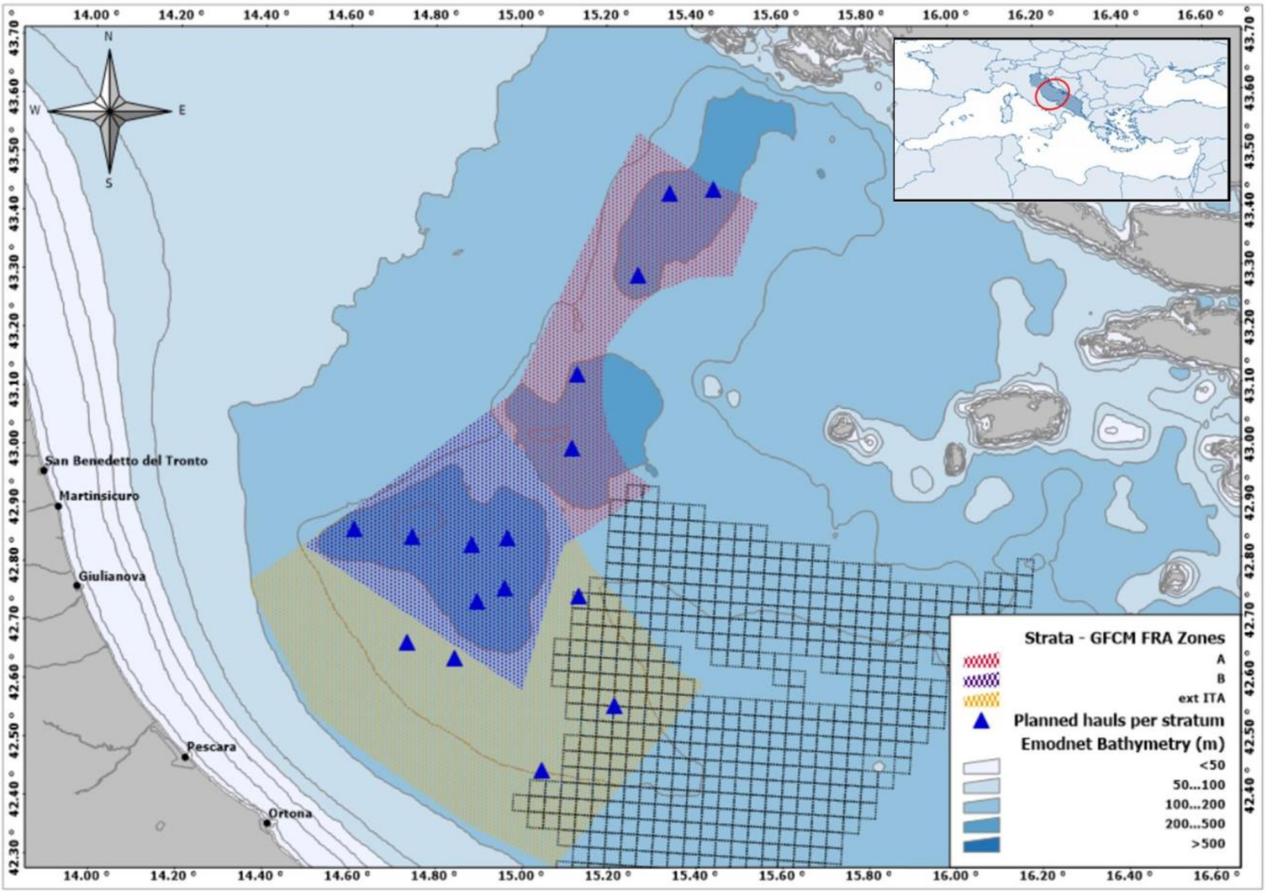

Figure 1. Study Area. In the up-right rectangle the position of the study area within the Mediterranean basis is highlighted (red circle). The main map shows: central Adriatic Sea bathymetry (source: [55]) and stratification in use within this study (dotted areas; zone "A", "B" and "ext ITA"); position (triangles) of the trawl hauls considered within this study; main fishing area (chequered area) for the Italian fleet targeting Norway lobster and European hake according to Russo et al. (2018).

Ultimately, decades of exploitation of commercial stocks by bottom trawling had most likely contributed to changes in the demographic structure and in some biological parameters of the populations of commercial species resident in the Pomo/Jabuka Pits [79]. Hence, a correct management of the area could be very important for the conservation of many species, including those of high commercial interest (*M. merluccius*, *N. norvegicus*, *P. longirostris*) [3]. Therefore, the area has long been the subject of discussions regarding the possibility to establish here a fishing ban [80,81]. Starting from 2015 some protection measures have been implemented both by Italian and Croatian authorities (changing various times in area closed and restriction measures). From 26 July 2015 to 16 October 2016 a part of the Pomo Pits area (Fig. 2, panel a) was closed to Italian bottom trawlers [82,83]. Subsequently, most of the previously defined area was reopened to trawlers, but with precautionary measures (limited number of licences and fishing days), and a ban was established for each fishing activity for an area ([84,85]; Fig. 2, panel b). After the yearly italian seasonal fishery closure of 2017 [86], with another ministerial decree [87], a fishing ban for the Italian fleet from 1 September 2017 was established in two areas (one including the western Pit and another portion of sea located close to Croatian territorial waters; Fig. 2, panel c) and a ban from 1 September to 31 October 2017 in the area including Italian territorial waters. A limited number of fishing authorizations was released for the area closest to the Italian coast with a series of additional management measures (e.g. number of fishing days allowed for each vessel). Likewise, the Croatian authorities have intervened with specific regulations for the Croatian fleet and over the waters under their jurisdiction. Finally, in October 2017, the General Fisheries Commission for the Mediterranean (GFCM), established a FRA in the Pomo Pits area (in force from 1 September 2017 and initially until 31 December 2020), in order to contribute to the protection of vulnerable marine ecosystems and essential habitats for important demersal stocks in the Adriatic such as hake and Norway lobster [88]. The FRA is made up of 3 different zones: zone "A" closed to any fishing activity, zones "B" and "C" subject to fishery restrictions (Fig. 2, panel d). The FRA was ratified by the EU in 2019 (EU, 2019). With the Recommendation 44/2021 GFCM [89], the FRA "Pomo/Jabuka Pits" was made permanent. Therefore, altogether the aforementioned management measures result in a change in the intensity and distribution of the fishing effort exercised in the area over time.

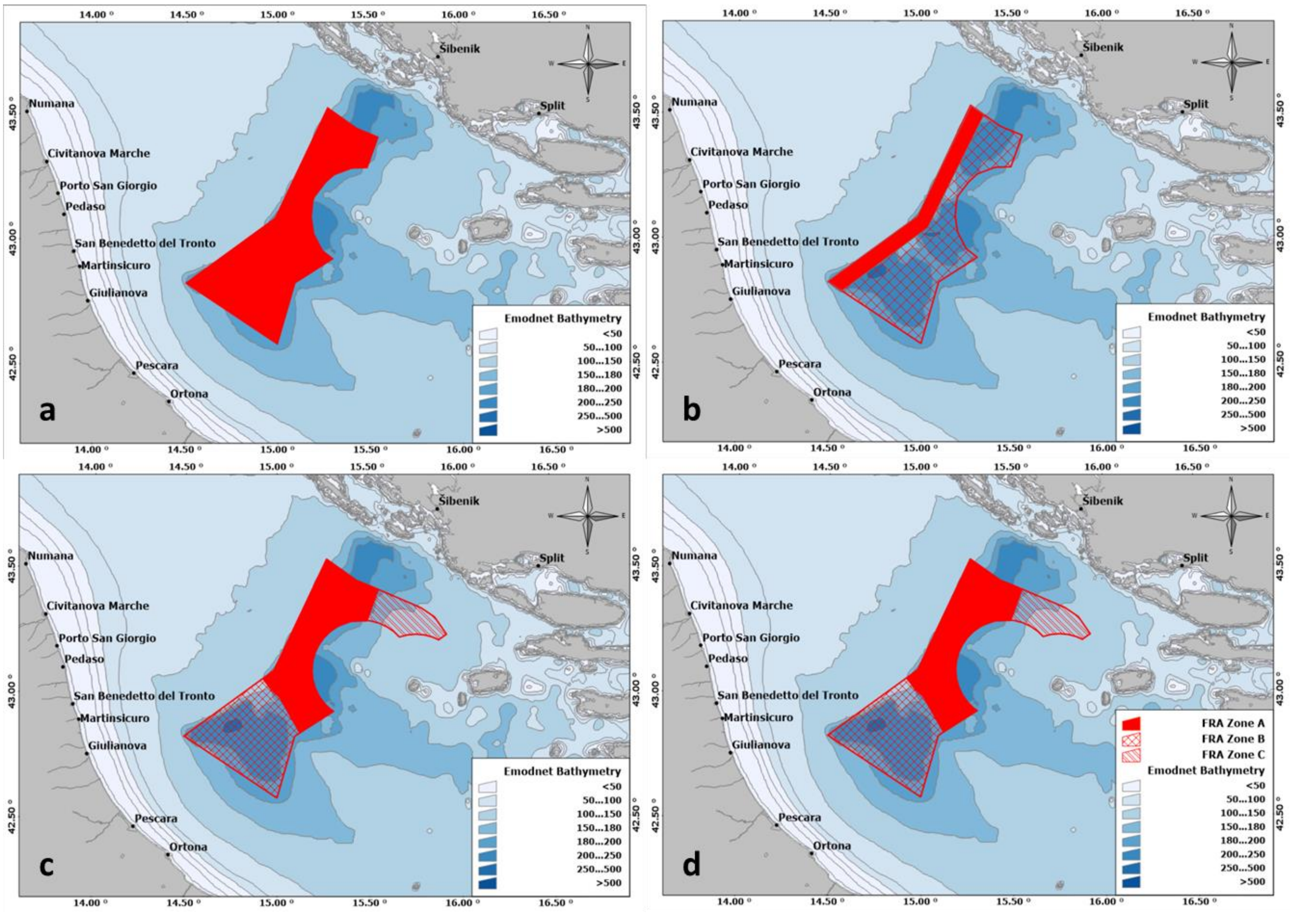

Figure 2. Management measures implemented in the Pomo Pits area since July 2015. Panel a shows (in red) the area closed to trawl fishery from 26/07/2015 to 16/10/2016, other types of fishing

activities such as longlines are permitted throughout the area. Panel b presents (in red) the area subjected to a ban to all fishing activities and an area (red sparse grid) where a limited number of licences and fishing days for trawlers are allowed from 01/10/2016 to 31/08/2017. Panel c reports (in red) the area closed to all fisheries from 01/09/2017 and the areas (red sparse grid) closed to all fishing activities until 31/10/2017 and then managed through special licences. Panel d refers to the establishment of a fishery restricted area: Zone "A" (in red) closed to all fisheries, zone "B" (red sparse grid) where the closure to fishing activities is from 01/09 to 31/10, than fishing is regulated with licences and with 2 days of permits per week (1 for the twin nets) and zone "C" (red sparse grid) where the closure to fishing activities is from 01/09 to 31/10, trawling is authorised through special licences on Saturdays and Sundays from 5.00 am to 10.00 pm, gillnets, pots and longines authorised, can fish there, from Monday at 5.00 am to Thursday at 22.00 pm.

The aim of this study is to present a simple alternative to the classic BACI analytical approach, applied in a rather complex framework as the "Pomo/Jabuka Pits" in the central Adriatic Sea, where changes in fishery management strategies were implemented since 2015; the proposed tool allowed the detection of signals, interpreted as early effects on the abundance of some commercially and ecologically important species.

2. MATERIAL AND METHODS

2.1 Sampling

From 2009 to 2019 (except for 2011 and 2018), the National Research Council - Institute of Marine Biological Resources and Biotechnologies (CNR-IRBIM) of Ancona (Italy), carried out jointly with the Institute of Oceanography and Fisheries (IOF) of Split (Croatia) an annual spring "UnderWater TeleVision" ("UWTV", hereafter referred to simply as spring) survey covering the entire area of the three meso-Adriatic depressions [56,90]. The survey was carried out under the auspices of the FAO – ADRIAMED project, from 2012 to 2016 it was also sponsored by the Italian National Flagship Program RITMARE and, for the experimental trawl fishery part (see below), from 2015 to 2018 also by the Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF). Originally, this fishery independent survey was aimed to quantify *N. norvegicus* burrows through video analysis of seabed footage [56], but meanwhile experimental trawling activities were also carried out in order to obtain additional demographic data on this and other important species inhabiting the area [72,91]; in this study, only catch data from these trawling activities are taken into account. Norway lobster is indeed a sedentary bottom-dweller that digs complex burrows in muddy sediments within which spends most of its lifetime [92]; animals inside or at the entrance of their burrows easily avoid capture by retracting themselves when trawl nets approach [93–95]. This burrowing behaviour heavily affects fishery leading to a high variability in catch, as an indication of fluctuation in the numbers of individuals undertaking emergence from their burrows [96–99]. Therefore, trawl hauls were always carried out at sunset and sunrise, corresponding to the maximum peak of emergence from burrows [100,101]. At the same time, these trawl hauls also provided information on the abundance and distribution (as well as length frequency distributions) of other commercially or ecologically relevant demersal species living in the area such as *P. longirostris*, *M. merluccius*, *Micromesistius poutassou*, and *Munida* spp [72,91]. Therefore the trawl data obtained by means of the spring surveys represent a very useful time series allowing comparisons between the period before fishery restriction implementation in the Pomo Pits area and the subsequent one [72]. Furthermore, following the enforcement of the first management measures in the area, within a series of agreements with the Italian the Ministry of Agricultural, Food and Forestry Policies (MIPAAF), in 2015 CNR-IRBIM of Ancona started an additional autumn trawl survey (namely the "ScamPo" survey, hereafter referred to simply as autumn survey) covering the western Pit and a larger buffer area [72]. In order to obtain data comparable with those collected before the management measures implementation, the same standard procedures used during the spring surveys were adopted and all cruises were carried out on board the RV Dallaporta (LOA 35.30 m, 258 GT, 1100 HP). Both surveys occurred in a consistent time period each year: April, May for the spring survey and September, October, November for the autumn survey; the same trawling protocol and general sampling design were adopted in overlapping areas (Fig 1; [72,91]). In particular, haul duration was fixed at 1 hour, starting almost half an hour before the sunset/sunrise and since 2012 all halus were conducted by means of the same experimental net (22 mm mesh size in the body and 12 mm in the cod end) and thus their catches can be compared over time; SIMRAD® trawl monitoring sensors were used to follow the fishing equipment behaviour during the hauls [72]. The net was equipped with two spread sensors on each wing end to measure the average horizontal opening; a trawl-eye sensor was used to monitor the net while approaching the bottom. It was thus possible to record the real time of starting/ending of the haul and the vertical opening of the net. Through a GPS system, the positions of the ship during the fishing operations were recorded minute by minute; the ship's position was then used as a proxy of the net's position for the purpose to calculate the effective swept area [72]. The total catch of each haul was weighed and in case of very high weights, a representative sub-sampling was carried out in order to allow the correct reconstruction of the total catch by species; all organisms, and in particular the principal species of commercial or ecological interest, were identified at the lowest possible taxonomic level, weighed and counted [72]. Norway lobsters and pink shrimps were divided by sex, the carapace length was recorded for each collected specimen at the lower mm using a caliper. For hake and blue whiting the total length of each individual was measured, at the lower half cm, by means of a graduated splint. For all the other species including *Munida* spp. the total weight and the total number of individuals were recorded.

2.2 Data analysis

All the collected information was entered in a database built by means of the Geographic Information System (GIS) Manifold® System Release 8 (http://www.georeference.org/doc/manifold.htm), through which the GPS data were verified and then processed in order to calculate the swept area of each haul [72]. The swept area was calculated by multiplying the distance covered by the net on the seabed and the average value of the net mouth opening for each haul. Finally, Catch-per-unit-of-effort (CPUE) estimates for *N. norvegicus*, *P. longirostris*, *M. merluccius*, *M. potassou*, *Munida spp.* (as well as other species), were calculated as total weight of the caught individuals divided by swept area (kg/km^2; hereafter referred to as biomass index) and number of caught individuals divided by the same swept area (N/km^2; hereafter referred to as density index), according to Martinelli et al. [72].

Considering data from 2012 onward, in order to assess the CPUE response as a potential effect of the management measures implemented over time and space, the possibility of *a-posteriori* applying a variant of the classic "before-after-control-impact" model design (BACI [44,45,102]) was explored. The considered variant could be summarised as a BIAMS (Before/Intermediate/After Multiple Sites) approach.

In order to perform this, three different strata (or sites) to be tested were *a-posteriori* selected within the study area: "A", "B", and "ext ITA" (Fig. 1). The adopted stratification follows indeed the "A" and "B" FRA' zones, where fishing effort levels changed in time according to the implemented management measures, and an additional buffer external area, "ext ITA", adjacent to zone "B" and located to the south-west, where no fishery limitations were implemented (except for the yearly seasonal trawl fishery closures regulated by national governments). Even if the peculiar bathymetry and oceanography of the area do not actually allow to *a-posteriori* define a proper "control" (i.e. having the same bathymetry compared to the other impacted areas [103]), the "ext ITA" area was meant to serve as a comparison with grounds where fishing activity was limited or banned through time and space. It was thus used to build an analytical spatial framework for a modified BACI analysis with the aim to evaluate the management measures performances. According to the original sampling design, the number of available haul *per* area is 5 in strata "A" and "ext ITA", and 6 in "B". Unfortunately, not all the planned hauls were performed every year due to technical issues such as limited ship availability, bad weather conditions etc. From 2012 to 2019 (except 2018) an average of 7.2 ± 1.3 stations were sampled during the spring surveys in the three considered areas, while from 2015 to 2019 an average of 6.6 ± 2.5 hauls were carried out during the autumn surveys.

In addiction, the temporal variations of the management measures from 2015 to 2018 (and their limited duration over time) further complicated the verification of their short / medium / long-term effects. Therefore, a temporal dimension was added to the the proposed modified BACI framework, following the scheme type "BEFORE / INTERMEDIATE / AFTER" defined as: i) the period prior to the implementation of the first management measures in 2015 (BEFORE, from 01/01/2012 to 01/07/2015); ii) the intermediate stage in which management measures have changed over time following the application of two decrees ([82,85] INTERMEDIATE, from 02/07/2015 to 31/08/2017); iii) after the application of the latest decree [87], thus considered as a period of application of the measures relating to the FRA until the end of 2019 (AFTER, from 01/09/2017 to 01/01/2020). Each of these time steps were considered as the level of the *Closure* factor, which therefore includes all the fisheries management measures adopted in the study area from 2012 to 2019.

The examination of fish response to protection measures in terms of biomass and length could be hampered by small sample sizes [13]. Hence, in the aim to consider a big enough sample size for the subsequent significance testings, the possibility of aggregating the data of the two cruises was explored. First the homogeneity of variance was assessed by a Levene test. In case of homoscedasticity, a classic t-test was applied on both CPUE (i.e. biomass and density indices). In case of heteroscedasticity, the t-test applied was that of Welch. The preliminary t-tests were applied on the biomass and density indices for each of the target species and considering areas "B" and "ext ITA" (area "A" was not considered because it was sampled only during the spring surveys); the t-tests were developed to assess a possible difference between the CPUE for the two cruises (i.e. spring and autumn surveys). In case of no difference, data from the two cruises could be pooled.

Afterwards, for each of the target species, in order to assess the effect of the *Closure* factor on the CPUE for each considered area ("A", "B" and "ext ITA") the following analyses were performed: i) a Levene test to verify the homogeneity of variances; ii) a parametric one-way analysis of variance (ANOVA); iii) the appropriate pairwise post hoc tests (according to Tukey, in case of homoscedasticity, or according to Games-Howell, in case of heteroscedasticity). The reference p value to determine significance was set at 0.01 (p values < 0.05 and > 0.01 were considered marginally significant). All the statistical tests and the relative graphics were made using the statistical software R [104,105] with associated packages car, ISwR, rpart, ggplot [106–109].

3. RESULTS

3.1 t test between the spring and autumn surveys

The Levene's tests conducted on the abundance indices of the two scientific surveys indicated homogeneity of the variance in all cases, only the biomass index for *P. longirostris* from area "B" showed marginal significance level (F test = 5.180, df = (1, 41), p-value = 0.029). The results of the t test for the biomass index were significant only for *M. merluccius* both in area "B" (t = -2.3183, df =

41, p-value = 0.026) and "ext ITA" (t = -2.2007, df = 22, p-value = 0.039). For the density index, the only significant difference was that for *M. poutassou* in area "ext ITA" (t = 2.8584, df = 14.167, p-value = 0.0126). Since only three marginal significant differences were observed between the two cruises out of twenty tests, in the following analyses the catch data of the two surveys were considered as belonging to a single data population and used as an aggregate for the subsequent ANOVA analyses.

3.2 One-way ANOVA for biomass and density indices by *Closure* factor for each stratum (A, B, ext ITA)

The one-way ANOVA generated several significant results, indicating that the *Closure* factor had an effect on both the CPUE indices in each of the strata (Tables S1-S5). The results of the post hoc pairwise comparisons, are summarised in Fig. 4 and here below:

a) Stratum A

For *M. poutassou* and *Munida spp.*, there is no evidence of an effect of the *Closure* factor on both CPUE (Fig. 4a). For *N. norvegicus* there is a strong evidence that the two indices were higher for the level "AFTER" of the *Closure* factor if compared to "BEFORE" (biomass index p-value = 0.001, density index p-value = 0.006); moreover, there is also a very marginal evidence that the biomass index is higher for the *Closure* level "AFTER" when compared to "INTERMEDIATE" (p-value = 0.043). For *M. merluccius*, there is a strong evidence that the two indices were higher when "AFTER" was compared to "BEFORE" (biomass index p-value < 0.001, density index p-value < 0.001) and to "INTERMEDIATE" (biomass index p-value = 0.01, density index p-value = 0.007) even if, in the case of biomass index, this last evidence is marginal. For *P. longirostris*, there is a strong evidence that both indices were higher when "AFTER" was compared to "BEFORE" (biomass index p-value < 0.001, density index p-value = 0.002) and were also higher when "INTERMEDIATE" was compared to "BEFORE" (biomass index p-value = 0.003, density index p-value = 0.004).

b) Stratum B

No evidence of difference was observed for the CPUE indices of *N. norvegicus*, *M. poutassou* and *Munida spp.*, Fig. 4b. For *M. merluccius*, there is only marginal evidence that the biomass index is higher when "AFTER" is compared to "INTERMEDIATE" (p-value = 0.03). For *P. longirostris*, there is evidence, marginal for biomass index and strong for density index, that both were higher when "AFTER" was compared to "BEFORE" (biomass index p-value = 0.022, density index p-value < 0.001); both indices were also higher when "INTERMEDIATE" was compared to "BEFORE" but in this case the evidence was marginal (biomass index p-value = 0.021, density index p-value = 0.022).

c) Stratum ext-ITA

For *M. merluccius* and *P. longirostris*, there was no evidence of a *Closure* effect, Fig. 4c. For *N. norvegicus*, there is marginal evidence that both indices were lower when "AFTER" was compared to "BEFORE" (biomass index p-value = 0.047, density index p-value = 0.016). For *M. poutassou*, there is only marginal evidence that the biomass index is lower when "BEFORE" was compared to "INTERMEDIATE" (p-value = 0.027). For *Munida spp*., there is only marginal evidence that the biomass index is higher when "INTERMEDIATE" is compared to "BEFORE" (p-value = 0.037).

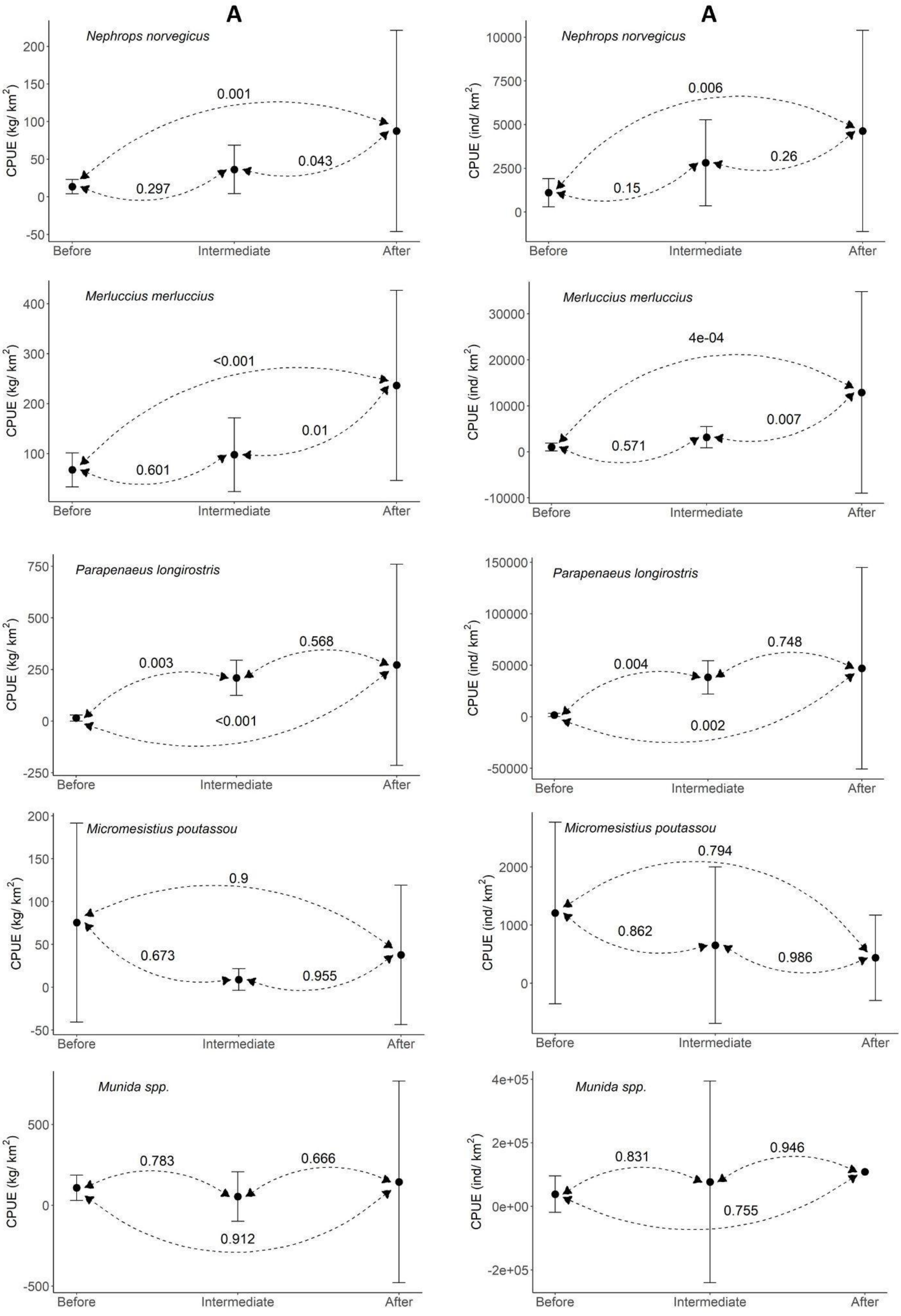

Figure 4a. Plot of catch for unit effort (CPUE) by species (rows) and type of index (columns) as a function of the *Closure* factor levels (BEFORE, INTERMEDIATE, AFTER) for stratum "A". Points and error bars represent means and 95% confidence intervals, respectively. Dotted arrows indicate the post hoc pairwise comparisons and the number above is the respective p-value.

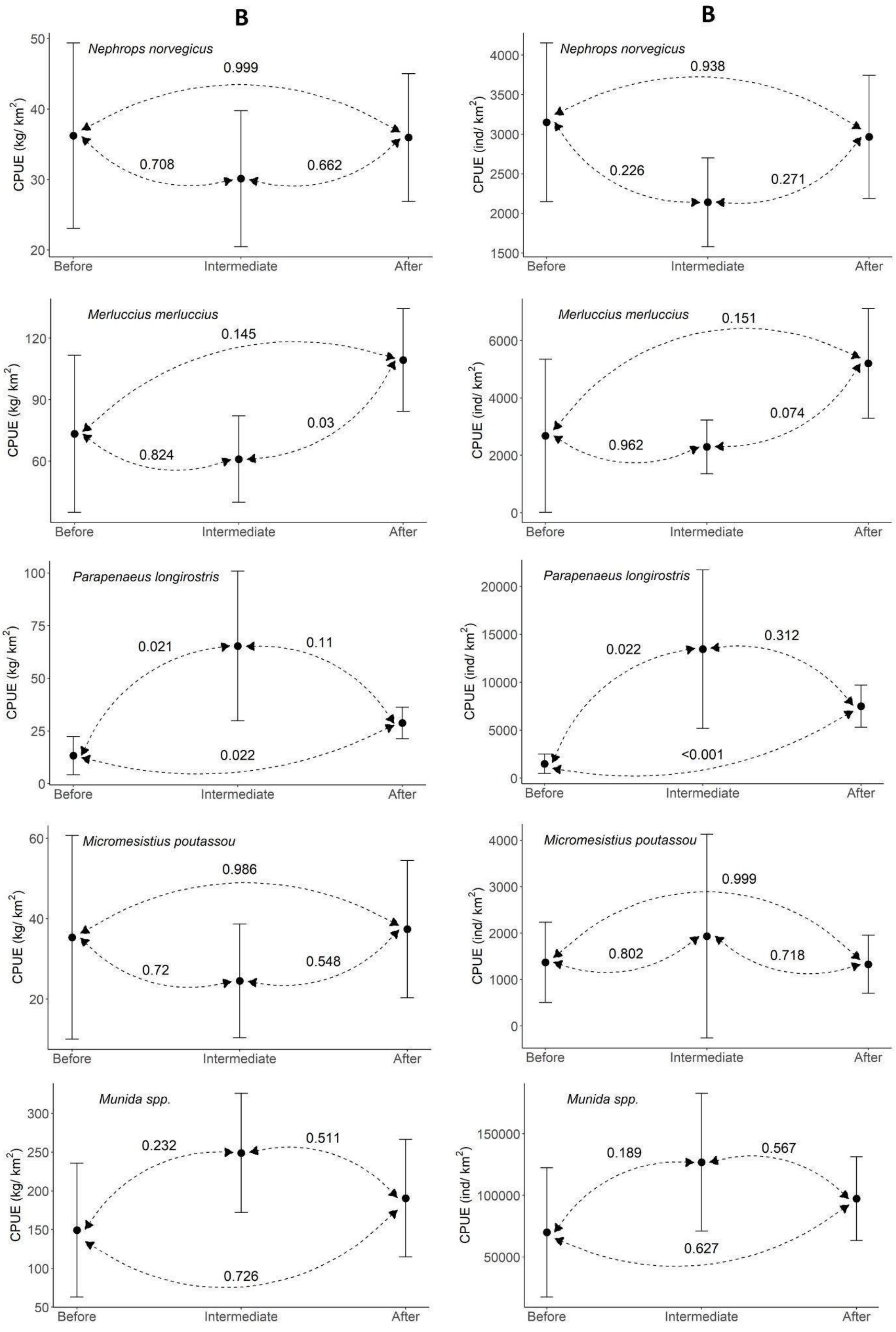

Figure 4b. Plot of catch for unit effort (CPUE) by species (rows) and type of index (columns) as a function of the *Closure* factor levels (BEFORE, INTERMEDIATE, AFTER) for stratum "B". Points and error bars represent means and 95% confidence intervals, respectively. Dotted arrows

indicate the post hoc pairwise comparisons and the number above is the respective p-value.

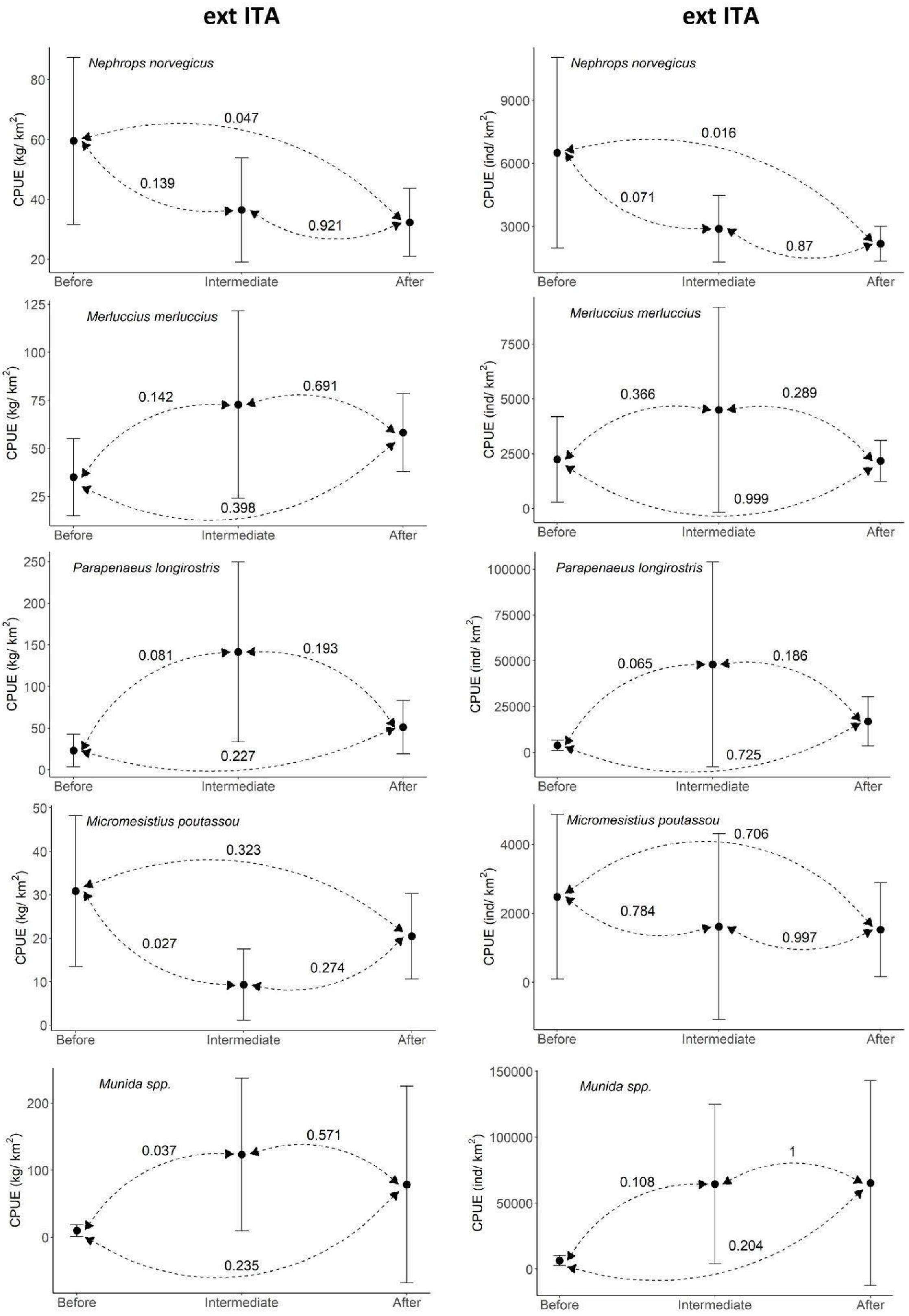

Figure 4c. Plot of catch for unit effort (CPUE) by species (rows) and type of index (columns) as a function of the *Closure* factor levels (BEFORE, INTERMEDIATE, AFTER) for stratum "ext

ITA". Points and error bars represent means and 95% confidence intervals, respectively. Dotted arrows indicate the post hoc pairwise comparisons and the number above is the respective p-value.

4. DISCUSSION

4.1 General framework and BIAMS application

Fishing activities and changes in fishing pressure may cause relevant variations in abundance indices [3,110]. On the other hand, Petza et al. [18] reported how valid the establishment of a FRA could be for managing fish resources. The Pomo/Jabuka Pits area was subject to numerous changes in time and space of the management measures adopted since 2015; these changes eventually resulted in the establishment of the currently in place FRA.

The time series produced by the two surveys conducted by the CNR-IRBIM of Ancona within the Pomo/Jabuka Pits area represent a reliable data series crossing the various management measures implemented from 2015 to 2019. In order to investigate possible discrepancies over time and space between their response to the considered management changes, within this study, both available abundance indices (i.e. kg/km^2 and N/km^2) were considered for five commercially and/or ecologically important species as: *N. norvegicus*, *M. merluccius*, *P. longirostris*, *M. Poutassou* and *Munida spp*. In fact, for example, for species such as *N. norvegicus* there could be possible differences between trends due to the fact that density may be size dependent [111].

In this study, a variant of the classic BACI analysis, defined as BIAMS, was adopted to assess the early effect of different management regimes implemented over time and space on some commercially and ecologically important species in the complex framework of the Pomo/Jabuka Pits' FRA establishment. Indeed, the various changes in protection levels in space and time from 2015 (when fishery management measures were implemented for the first time) until 2017 (when a more stable management regime was applied) and the absence of an available and previously defined proper control site do not allow the application of traditional and user-friendly tools as the classic BACI approach. Therefore, with the aim of building an appropriate, but at the same time easy to use and able to give an intuitive depiction, analytical spatial and temporal framework to evaluate the impact of the management measures implemented in the area and in particular the early effects of the FRA, a modified BACI approach was proposed.

More specifically three zones *a posteriori* stratification was introduced in the analysis. The adopted stratification was partially based on the spatial extent of the zones defined in the GFCM/41/2017/3 recommendation (Fig. 2) [88]. These zones were designed primarily for the recovery and maintenance of fish stocks through the identification of EFHs, not taking into account issues linked to future evaluations of their possible effects. Furthermore, the spatial boundaries mainly followed the bathymetry of the area, resulting in the absence of an adequate control site. Therefore, the adopted *a posteriori* stratification, including zone A and B (defined by the FRA) and external buffer area (i.e. "ext ITA") intended as a proxy control site, was also meant to fulfil this lack by identifying a stratum in which no specific management measures were implemented, to be compared with grounds where, since 2015, fishing activity was limited or banned. In addition, the use of 3 adjacent strata, instead of geographically independent sites may allow the detection of possible interactions among areas for mobile species (e.g. movement of fishes from a nursery to adjacent grounds).

Furthermore, in this study a multi level temporal dimension was added to the classical 2 steps BACI framework including 3 levels for the *Closure* factor, in order to allow comparisons of the period in which no fishery restrictions were in place ("BEFORE"), with the following in which starting from 2015 management measures were implemented and modified in time and space various times ("INTERMEDIATE"), and with the last time lag starting in 2017 when fishing activity was regulated in a more stable way ("AFTER"). The choice of these time intervals was made in accordance with regulations that followed each other (Fig. 2). Globally, the period considered within this study for the BIAMS approach application ranges from 2012 to 2019, therefore the three adopted time steps are relatively short and allow to detect only early effects of changes in management measures [22,23]; this is especially true for the last considered time step relative to the establishment of the FRA regime, which is still in place nowadays. However, in future studies this scheme could be implemented in order to assess possible medium or long-term effects, thus it would be important to carry out more surveys and maintain the time series.

4.2 Detected early effects of the implemented management measures on abundance indices

No particular differences were observed in the response of the two different CPUE (i.e. biomass and density indices) for each strata and species combination. The only exceptions were found in the “ext ITA” stratum for *M. poutassou* and *Munida spp.* in the "BEFORE-INTERMEDIATE" comparison, as well as in the “B” stratum for *M. merluccius* in the “INTERMEDIATE-AFTER” comparison both resulting in a marginal significance of the biomass indices not reflected in a significance of the respective density indices.

For *N. norvegicus*, *M. merluccius* the mean CPUE, for both biomass and density indices, in area "A" showed a gradual increase over the three different levels of the *Closure* factor. These results suggest that the establishment first of some management measures in 2015 and then of a stable no-take zone in area "A" since October 2017, had positive effects on the target species. These findings are in agreement with those described by Martin et al. [112] in northern Spain, where a decrease in fishing effort positively influenced the populations of *N. norvegicus and M. merluccius* present in the area.

Following the establishment of the first management measures from 2015 to 2017 (i.e. "INTERMEDIATE-AFTER") significant positive effect on biomass indices resulted only for *N. norvegicus* and *M. merluccius* suggesting an early positive effect probably due to the implementation of the first fishing bans. For *P. longirostris* this positive effect was not significant, while both the indices in the "BEFORE-INTERMEDIATE" comparison showed a significant increase suggesting that the increase in the mean CPUE could be related not only to the implementation of management measures, but also to other variables such as possible changes in environmental conditions (e.g. temperature and/or salinity) that may favour this species [70,71]. In fact, effects of salinity on spawning of this species and of temperature on its catch rates were already reported in literature [73]. The mean CPUE for *Munida spp.* in stratum “A” over the fishery *Closure* levels were quite stable, hence the establishment of a fishery ban in the area appears to have no evident effects on this species.

In area "B" where the fishery was subjected to a series of limitations since 2015, the only significant effect resulted for *P. longirostris* in accordance with the "BEFORE-AFTER" comparison for area "A". It is interesting to note that in this area the mean CPUE for *M. merluccius* increased after the application of the first management measures (i.e. "INTERMEDIATE-AFTER") but it is marginally significant only for the biomass index; while for the remaining species the mean CPUE is more variable even by comparing the two indices for the same species. Hence the establishment of solely fishing restrictions may not be sufficient for an actual increase in CPUE in a relatively short time period for the target species. However, in agreement with what reported by Roberts et al. [4] and Batista et al. [113], the occurrence of a significant effect on the CPUE of a mobile species such as *M. merluccius* in the area adjacent to the closed one could be attributable to a potential spillover effect as a consequence of the significant increase in mean observed CPUE in area "A" following the establishment of the fishery ban.

Instead, in the "ext ITA" area the mean CPUE of *N. norvegicus* for both indices decreased and this may suggest that the absence of management measures in this area actually affects the CPUE for this species over time. However, no such a sharp decrease in mean CPUE was observed for *M. merluccius*, but the values were very similar to these recorded before the establishment of the first fishery management measures (i.e. "BEFORE-AFTER"). This equilibrium condition may have been reached as a result of a combined effect between the possible spillover effects and the displacement of the fishing effort within the areas adjacent to the FRA. Indeed, as reported in Bastardie et al. [3], a displacement of the fishing effort from the area where fishery ban or restrictions were implemented (i.e. "A" and "B") to areas without fishery limitations (i.e. "ext ITA") could affect the CPUE. It would be thus interesting to include in future evaluation of the FRA impact also the possible variations in the distribution of fishing effort estimated by means of Vessel Monitoring Systems [114], Automatic

Identification System [115,116] or a combination of both [117].

As previously stated, the catch rates of some commercial species such as *P. longirostris* could be favoured by possible changes in environmental conditions which may thus mask the positive effect on CPUE due to the implementation of fishery management measures. According to Marini et al. [58] the Pomo/Jabuka Pits area is characterized by a peculiar oceanographic regime which can affect the status of marine communities [59]. Therefore, also thanks to the presence of relevant time series of oceanographic data specific for the study area [118,119], future studies aimed to investigate and possibly quantify the combined effect of the FRA related to changes in environmental conditions, as well as to detect possible long-term effects, should be carried out. It could be achieved through more complex approaches as already trialled for *N. norvegicus* by Chiarini et al. (submitted). Furthermore, next actions may be focused to integrate in this evaluation the length frequency distribution as indicator allowing to detect the possible effects of management regimes on the different length classes.

5. CONCLUSION

The overarching goal of this study was to perform a short-term evaluation from 2012 to 2019 of the effects on some commercially or ecologically important species of changes in fisheries management measures implemented within the Pomo/Jabuka Pits area since 2015 (Adriatic Sea). The variant of the classical BACI analysis here proposed, and called BIAMS, showed globally a positive effect on the target species with a significant increase in biomass and density indices in particular following the establishment of a no-take zone in stratum "A". With regards to areas subjected to fishery limitations (i.e. area "B"), the analysis did not show a significant increase in average CPUE. However, a probable spillover effect from the no-take-zone was evidenced for *M. merluccius*. Furthermore, for *P. longirostris* a significant increase in the average CPUE for both indices was observed regardless of the adoption of the management measures; this could be thus related also to changes in environmental conditions which are known to affect this species (e.g. temperature, salinity). The mean CPUE, in particular for *N. norvegicus*, suffered a decrease in the stratum that was never subject to particular fishery limitations and adjacent to the FRA (i.e. "extITA"); probably this can be attributed to a displacement of the fishing effort following the implementation of the management measures. To be noticed that instead for species such as European hake this decrease is less evident in the same stratum; the reason behind this could be identified in a combined effect of the spillover and the displacement of the fishing effort which possibly led to a balance on the average CPUE. Hence, even if it is a rather simple tool when compared to other powerful approaches, the here proposed BIAMS, allowed to easily identify a series of early effects of the implementation of different management measures over time and space in a complex and very relevant framework (i.e. the Pomo/Jabuka Pits area), lacking a proper independent control site and having a complicated temporal dimension.

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SUPPLEMENTARY MATERIALS

S1 Table. Parametric one-way ANOVA for biomass (kg/km^2) and density (N/km^2) indices of *Nephrops norvegicus* by *Closure* factor for strata "A", "B" and "ext ITA". Analysis of the variance of both CPUE indices (biomass index above, density index below) by area ("A", "B", "ext ITA") across the three levels of the *Closure* factor for *Nephrops norvegicus*. "*Closure*" is the factor, "Df" is the number of degree of freedom, "Sum Sq" represents the sums of squares, "Mean Sq" is the mean of squares, "F" is the ratio of the sum of squares, "Pr(>F)" is the combination of the F-statistic with the degrees of freedom. The asterisk (*) refers to the level of significance.

CPUE index	**Area**		**Df**	**Sum Sq**	**Mean Sq**	**F**	**Pr(>F)**
Biomass index	"A"	*Closure*	2	12740	6370	10.35	0.00174**
		Residuals	14	8616	615		

	“B”	Closure	2	305	152.7	0.452	0.64
		Residuals	40	13512	337.8		
	“ext ITA”	Closure	2	3292	1646.0	3.507	0.0485*
		Residuals	21	9855	469.3		
Density index	“A”	*Closure*	2	31266327	15633163	7.44	0.00629**
		Residuals	14	29416444	2101175		
	“B”	Closure	2	7105754	3552877	1.709	0.194
		Residuals	40	83146953	2078674		
	“ext ITA”	Closure	2	82906158	41453079	4.9917	0.01684*
		Residuals	21	174391574	8304361		

S2 Table. Parametric one-way ANOVA for biomass (kg/km^2) and density (N/km^2) indices of *Merluccius merluccius* by *Closure* factor for strata “A”, “B” and “ext ITA”. Analysis of the variance of both CPUE indices (biomass index above, density index below) by area (“A”, “B”, “ext ITA”) across the three levels of the *Closure* factor for *Merluccius merluccius*. “*Closure*” is the factor, “Df” is the number of degree of freedom, “Sum Sq” represents the sums of squares, “Mean Sq” is the mean of squares, “F” is the ratio of the sum of squares, “Pr(>F)” is the combination of the F-statistic with the degrees of freedom. The asterisk (*) refers to the level of significance.

CPUE index	**Area**		**Df**	**Sum Sq**	**Mean Sq**	**F**	**Pr(>F)**
Biomass index	“A”	*Closure*	2	66005	33002	11.99	0.000925***
		Residuals	14	38541	2753		
	“B”	Closure	2	20115	10057	4.055	0.0249*
		Residuals	40	99204	2480		
	“ext ITA”	Closure	2	5102	2551	2.007	0.159
		Residuals	21	26692	1271		

Density index	“A”	*Closure*	2	326475378	163237689	13.12	0.000617***
		Residuals	14	174184408	12441743		
	“B”	Closure	2	80081735	40040867	3.223	0.0504
		Residuals	40	496990709	12424768		
	“ext ITA”	Closure	2	26287903	13143952	1.412	0.266
		Residuals	21	195474073	9308289		

S3 Table. Parametric one-way ANOVA for biomass (kg/km^2) and density (N/km^2) indices of *Parapeneus longirostris* by *Closure* factor for strata “A”, “B” and “ext ITA”. Analysis of the variance of both CPUE indices (biomass index above, density index below) by area (“A”, “B”, “ext ITA”) across the three levels of the *Closure* factor for *Parapeneus longirostris*. “*Closure*” is the factor, “Df” is the number of degree of freedom, “Sum Sq” represents the sums of squares, “Mean Sq” is the mean of squares, “F” is the ratio of the sum of squares, “Pr(>F)” is the combination of the F-statistic with the degrees of freedom. The asterisk (*) refers to the level of significance.

CPUE index	**Area**		**Df**	**Sum Sq**	**Mean Sq**	**F**	**Pr(>F)**
Biomass index	“A”	*Closure*	2	209287	104644	16.42	0.000213***
		Residuals	14	89224	6373		
	“B”	Closure	2	16984.39	8492.2	8.27	0.001
		Residuals	40	41096.47	1027.41		
	“ext ITA”	Closure	2	54724.67	27362.33	5.62	0.011
		Residuals	21	102328.02	4872.76		
Density index	“A”	*Closure*	2	6.823e+09	3.411e+09	13.85	0.000481***
		Residuals	14	3.449e+09	2.463e+08		

	“B”	Closure	2	821385025.13	410692512.56	7.17	0.002
		Residuals	40	2290477144.48	57261928.61		
	“ext ITA”	Closure	2	7.267e+09	3.634e+09	3.035	0.0695
		Residuals	21	2.514e+09	1.197e+09		

S4 Table. Parametric one-way ANOVA for biomass (kg/km^2) and density (N/km^2) indices of *Micromesistius poutassou* by *Closure* factor for strata “A”, “B” and “ext ITA”. Analysis of the variance of both CPUE indices (biomass index above, density index below) by area (“A”, “B”, “ext ITA”) across the three levels of the *Closure* factor for *Micromesistius poutassou*. “*Closure*” is the factor, “Df” is the number of degree of freedom, “Sum Sq” represents the sums of squares, “Mean Sq” is the mean of squares, “F” is the ratio of the sum of squares, “Pr(>F)” is the combination of the F-statistic with the degrees of freedom. The asterisk (*) refers to the level of significance.

CPUE index	**Area**		**Df**	**Sum Sq**	**Mean Sq**	**F**	**Pr(>F)**
Biomass index	“A”	*Closure*	2	13506	6753	0.395	0.681
		Residuals	14	239193	17085		
	“B”	Closure	2	1308	654.2	0.583	0.563
		Residuals	40	44895	1122.4		
	“ext ITA”	Closure	2	1626	812.8	3.983	0.0342*
		Residuals	21	4286	204.1		
Density index	“A”	*Closure*	2	1800852	900426	0.279	0.76
		Residuals	14	45128323	3223452		
	“B”	Closure	2	3034463	1517231	0.334	0.718
		Residuals	40	181639265	4540982		
	“ext ITA”	Closure	2	4236799	2118399	0.36	0.702

		Residuals	21	123596520	5885549		

S5 Table. Parametric one-way ANOVA for biomass (kg/km^2) and density (N/km^2) indices of *Munida spp*. by *Closure* factor for strata "A", "B" and "ext ITA". Analysis of the variance of both CPUE indices (biomass index above, density index below) by area ("A", "B", "ext ITA") across the three levels of the *Closure* factor for *Munida spp*. "*Closure*" is the factor, "Df" is the number of degree of freedom, "Sum Sq" represents the sums of squares, "Mean Sq" is the mean of squares, "F" is the ratio of the sum of squares, "Pr(>F)" is the combination of the F-statistic with the degrees of freedom. The asterisk (*) refers to the level of significance.

CPUE index	**Area**		**Df**	**Sum Sq**	**Mean Sq**	**F**	**Pr(>F)**
Biomass index	"A"	*Closure*	2	15243	7622	0.406	0.674
		Residuals	14	262503	18750		
	"B"	Closure	2	58295	29148	1.412	0.256
		Residuals	40	825656	20641		
	"ext ITA"	Closure	2	32482	146241	4.336	0.0409*
		Residuals	11	41203	3746		
Density index	"A"	*Closure*	2	5.060e+09	2.530e+09	0.347	0.722
		Residuals	5	3.641e+10	7.282e+09		
	"B"	Closure	2	1.861e+10	9.307e+09	1.596	0.216
		Residuals	37	2.157e+11	5.830e+09		
	"ext ITA"	Closure	2	13484092608.82	6742046304.41	4.36	0.036
		Residuals	13	20097465115.76	1545958855.06		

4.2 Accounting for environmental and fishery management factors when standardising CPUE data from a scientific survey: A case study for *Nephrops norvegicus in the Pomo Pits area (Central Adriatic Sea)*

Abstract

Abundance and distribution of commercial marine resources are influenced by environmental variables, which together with fishery patterns may also influence their catchability. However, Catch Per Unit Effort (CPUE) can be standardized in order to remove most of the variability not directly attributable to fish abundance. In the present study, Generalized Additive Models (GAMs) were used to investigate the effect of some environmental and fishery covariates on the spatial distribution and abundance of the Norway lobster *Nephrops norvegicus* within the Pomo/Jabuka Pits (Central Adriatic Sea) and to include those that resulted significant in a standardization process. N. norvegicus is a commercially important demersal crustacean, altering its catchability over the 24-h cycle and seasons according to its burrowing behavior. A historically exploited fishing ground for this species, since 2015 subject to specific fisheries management measures, is represented by the meso-Adriatic depressions, which are also characterized by particular oceanographic conditions. Both the species behaviour and the features of this study area influence the dynamics of the population offering a challenging case study for a standardization modelling approach. Environmental and catch data were obtained during scientific trawl surveys properly designed to catch *N. norvegicus*, thus improving the quality of the model input data. Standardization of CPUE from 2 surveys from 2012 to 2019 was conducted building two GAMs for both biomass and density indices. Bathymetry, fishing pressure, dissolved oxygen and salinity proved to be significant drivers influencing catch distribution. After cross validations, the tuned models were then used to predict new indices for the study area and the two survey series by means of informed spatial grids, composed by constant surface cells, to each of which are associated average values of environmental parameters and specific levels of fishing pressure, depending on the management measures in place. The predictions can be used to better describe the structure and the spatio-temporal distribution of the population providing valuable information to evaluate the status of such an important marine resource.

Keywords: Adriatic Sea, *Nephrops norvegicus*, CPUE Standardisation, GAM, environmental parameters, fisheries management measures.

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norvegicus in the Pomo Pits area (Central Adriatic Sea). First submission: 02/08/2021; currently under a third round of revision after acceptance with major revision (last re-submission: 08/05/2022)

Introduction

Information about the distribution and abundance of marine species is usually derived from fishery-dependent data (e.g. sampling on board commercial vessels) or fishery-independent data (e.g. scientific surveys at sea) [1]. Catch Per Unit of Effort (CPUE), or catch rate, can be used as an index of abundance and it is the primary source of information for many of the most valuable and vulnerable commercial fishery resources [2] including several crustacean species [3,4]. However raw CPUE, intended as the total catch divided by an observable measure of associated effort, is rarely proportional to the real abundance of a resource over time and space [5], because numerous factors can affect catch rates [6]. Indeed, the CPUE index depends on the abundance of a resource, the fishing effort and also the catchability (intended as the fraction of an available resource that is captured by one unit of effort) [6,7]. Catchability can be assumed as constant or changes in catchability can be modeled and removed from the index [8]. Indeed, catchability is often spatiotemporally affected by environmental, biological, and management (such as fishery technological and/or management strategy changes) factors [2,9,10]. The process of removing the effect of factors that bias catch rate as an index of abundance is commonly referred to as CPUE standardization [11] and it allows obtaining more accurate estimates of abundance indices [12,13] and related standard errors [14,15]. In fact, fishery-dependent raw CPUE commonly vary over both space and time showing coverages mainly driven by fisheries patterns [16]. Even time series obtained from scientific surveys may suffer for gaps in space and time (e.g. missing years, sampling stations allocation, etc..) as well as for discontinuities in the sampling intensity or strategy (e.g. change of protocol, vessel, gear used). Therefore, it would be relevant to remove factors other than abundance that may influence CPUE variability before using an index as an indicator of population size [7,17].

Moreover, ecological processes (both exogenous and endogenous) can affect the species distribution and density [18–20]. Such a spatio-temporal dependence has been traditionally incorporated into CPUE standardization using spatial grids to predict the spatial distributions and relative abundances of marine species [e.g. 21,22]. Therefore, to accurately estimate the total population, a standardization process should first of all account for spatial and temporal differences in sampling rates, for example when the spatial pattern of the observation is not adequate [23] or when statistical dependence arises from biological or sampling characteristics (e.g shoals movement and extraordinary catch events) [24]. Second, the standardization should account for the effect of covariates which could have an impact on the catch rates over time and space [25]. Environmental conditions are among the factors that control the spatio-temporal distribution of fish populations, therefore it is crucial to identify their relationships with catch rates [26]. In addition, changes in fishing effort due, for example, to the establishment of a temporary fishing ban or a no-take zone, may result in changes of catch rates in time [27] and space (e.g. buffer areas [28,29]). However, the standardization of a catch rate time series must be designed to include only explanatory variables that significantly influence the dependent variable (i.e. CPUE [30]). Too few explanatory variables will cause variation in catch rate to be wrongly attributed to the time series, while too many explanatory variables will over-fit the model [11].

Historically, many efforts have been made to solve the difficulties associated with CPUE standardization [7] promoting the flexibility and the availability of well-tested and user-friendly tools such as Generalized Linear Models (GLMs) and Generalized Additive Models (GAMs) to perform calculation [31]. However, while considering the nonlinearity of predictors there is evidence that statistical models such as GAMs perform better than GLMs [32] even if the survey area is not well covered due to the lack of sampling locations or biased designs [33]. GAMs proved to be also helpful to understand the environmental processes underlying species distributions [34]. Furthermore, with the aim to produce robust abundance indices with associated standard errors [34], more sophisticated approaches such as Vector Autoregressive Spatio-Temporal (VAST) model and Boosted Regression Tree (BRT) were also recently implemented [35,36].

Within the Mediterranean basin, the Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758) (hereafter referred to by genus alone), represents one of the main commercial species in terms of value and is mainly targeted by bottom trawlers [37]. This demersal species lives at depths from around 30 m to over 400 m [38,39] with a preference for muddy grounds allowing the formation of its characteristic burrows [40]. This species is mainly captured by bottom trawlers when it emerges from its burrows [41–43]. Several studies provided evidence of a rhythmicity in the burrow emergence pattern showing peaks that vary in time depending on depth [44–46] and other ecological and demographic factors (i.e. food availability, size, sex, reproductive status [47–49]). Literature put in evidence how changes in environmental parameters, such as dissolved oxygen [40], salinity [50] and bottom temperature [51], proved to be fundamental drivers influencing life cycle and emergence behaviour of wild populations of *Nephrops*. Therefore, the emergence rhythm may cause marked fluctuations in CPUE over the 24-h cycle [39,52,53] and seasons depending on sex (females rarely leave their burrows during the egg-bearing period [40,54]), which means that the trawl fishery exploits the population selectively and in a different manner during the year. As suggested by Sardà and Aguzzi, accounting for such an availability of *Nephrops* to trawlers, not only according to the time of the day but also at a seasonal scale, may lead to more reliable estimates of population abundance [55]. Hence these differences in *Nephrops*' CPUE over different time scales (i.e. time of the day and season) should be considered directly within the standardization process, when possible.

In the Adriatic Sea, *Nephrops* is subjected to high fishing pressure from fleets of different countries [56]. Relevant concentrations of this species occur off Ancona, in the Pomo/Jabuka Pits and in the Velebit Channel [53,57–62]. There is evidence that *Nephrops* in the Adriatic Sea is also characterized by distinct subpopulations which should be considered as separate units [63,64] especially from the fishery management point of view [65]. According to a recent Scientific Technical and Economic Committee for Fishery report [66], in the Adriatic Sea this species is considered overexploited; the fishery-independent data used to implement this stock assessment derived from scientific surveys not specifically targeting *Nephrops*. However this type of data source was already defined not fully appropriate to overcome the general issues related to the catchability of this species, and the need for more detailed information from surveys specifically targeting this resource, in order to support an accurate *Nephrops* management, was already stated [65].

A sustainable use of fishery resources should be fostered by an ecosystem approach to fisheries management [67,68]. To move towards this, it is crucial to understand the impact of fisheries and environmental factors on benthic communities and their dynamics [69,70]. Angelini et al. developed an ecosystem model for the Pomo/Jabuka Pits and highlighted the need of improving it including environmental variables thus considering the influence of the hydrographic processes within the meso-adriatic depression on *Nephrops* distribution [71]. Furthermore, starting from 2015, the Jabuka/Pomo area has been the object of a series of management measures, which have greatly modified the spatial patterns of the fishing effort [28,72].

Focusing on data obtained from two annual dedicated *Nephrops* surveys carried out in the Pomo/Jabuka Pits, the present study proposes the use of GAMs as tools to: i) investigate how the environmental variables and the different management strategies can influence abundance and distribution of *Nephrops*, ii) produce standardized CPUEs, to be used as reliable inputs for population dynamic and ecosystem models.

Materials and Methods

The study area

The study area defined in Fig 1 is located in the Central Adriatic Sea and includes the three meso-Adriatic depressions collectively known as Pomo/Jabuka Pits, that altogether have a maximum depth of 270 m [73] and a seabed mainly composed by fine muddy substrates [74].

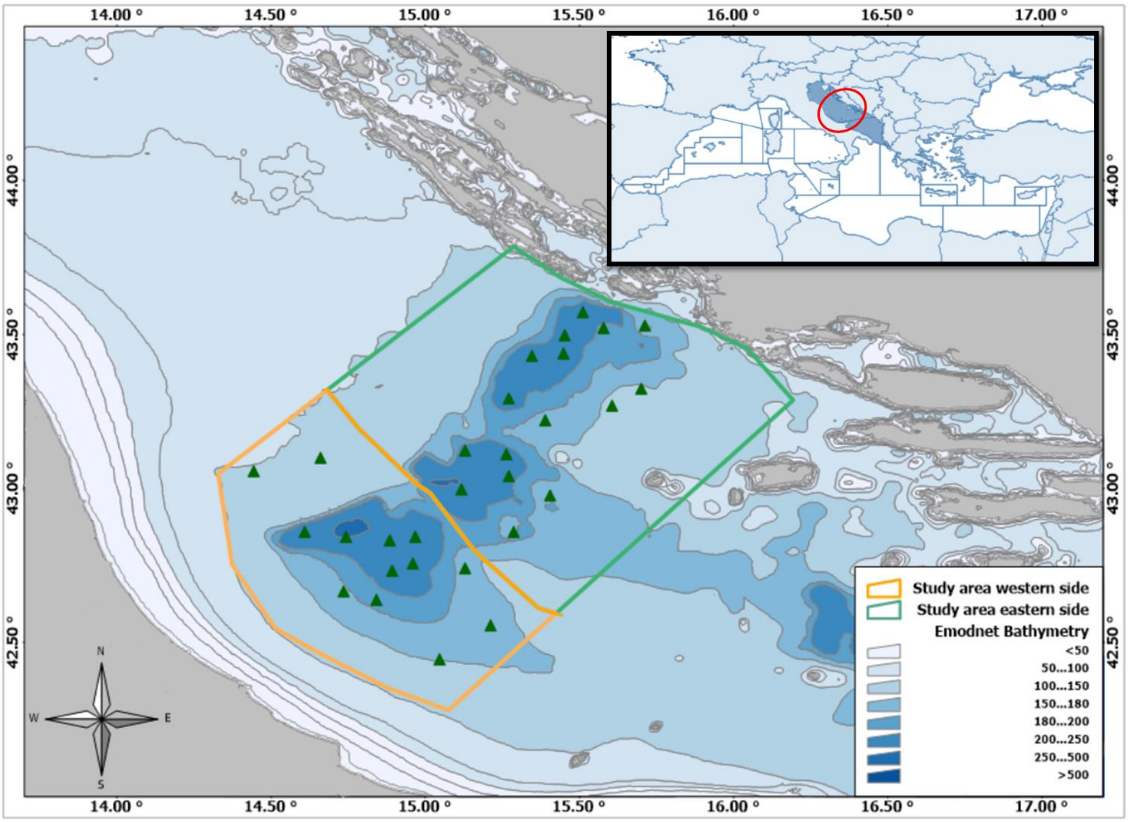

Fig 1. The study area. In the up-right rectangle the position of the study area within the Mediterranean basin (shape source: https://www.fao.org/gfcm/data/maps/gsas/fr/) is highlighted (red circle). The main map shows: the Pomo Pits Bathymetry (source: [75]), the boundaries of the study area, with western (orange polygon) and eastern (green polygon) sides divided by the Adriatic midline (source: [76]) and the trawl hauls planned for two considered surveys (green triangles).

The depth range and sediment composition make the Pomo/Jabuka Pits an ideal habitat for *Nephrops* [38–40] whose subpopulation is here characterized by high densities of individuals smaller than those dwelling in nearby areas (e.g. off Ancona) [57,63]. Furthermore, this area was also identified as a nursery ground for the European hake, *Merluccius merluccius* (Linnaeus, 1758), which is another important commercial demersal species in the Adriatic Sea [77,78]. Owing to the presence of essential habitats in a region exploited by both Italian and Croatian fisheries, the Pomo/Jabuka Pits was subjected to several discussions regarding the possibility of establishing an area closed to fisheries to protect heavily exploited demersal stocks [79,80]. The first partial closure of trawling activities was approved in July 2015 (D.M. 03/07/2015 and D.M. 20/07/2016; N.N. 20/07/2015 and N.N. 22/07/2016); then several decrees changed the fishing restrictions over time and space (D.M. 19/10/2016; D.D. 7/12/2016; D.M. 01/06/2017; N.N. 17/05/2017). Finally in 2018, a Fishery Restricted Area (FRA) was established identifying a fishery ban zone (S1 Fig, zone A) and two buffer zones where the fishing is limited to a specific number of authorized vessels and fishing days (S1 Fig, zones B and C) [81–83].

Data collection

The analyzed data originate from two fishery-independent surveys carried out annually within the Pomo/Jabuka Pits: (i) the spring "Under Water TeleVision" (UWTV) survey, performed jointly by the National Research Council - Institute of Marine Biological Resources and Biotechnologies (CNR IRBIM) of Ancona (Italy) and the Institute of Oceanography and Fisheries (IOF) of Split (Croatia) in the whole study area; (ii) the autumn "ScamPo" experimental trawl survey, carried out by CNR IRBIM of Ancona western of the Adriatic midline [84]. The "UWTV" (hereafter referred to as spring) survey principally aims to quantify *Nephrops* burrows through video analysis of seabed footage [85,86] but during this also experimental trawling activities are carried out [63,72,87]; these latter data on trawling were those considered in the context of this study. Instead, the "ScamPo" (hereafter referred to as autumn) survey is mainly meant to monitor the effects of the management measures on commercially relevant demersal species (including *Nephrops)* [72]. The time series considered within this study ranges from 2012 to 2019 for the spring surveys, and from 2015 to 2019 for the autumn ones.

As suggested by Kimura and Somerton, both surveys were designed to fulfill the basic assumption of maintaining standard procedures during trawl sampling (e.g. standard unit of time or distance) [88]. For this reason the surveys occurred in a consistent time period each year: late spring for "UWTV" (i.e. April, May) and autumn for "ScamPo" (i.e. September, October, November). The surveys were all carried out on board RV Dallaporta (LOA 35.30 m, 258 GT, 1100 HP). The 2 seasonal surveys share the same survey design (originally random stratified based on the bathymetric of 200 meters depth and following some legislative limitations) in the common area (i.e. western side) [72,87]; some additional hauls have been added in both survey plans since 2016 (S2 - S5 Figs) [72]. Unfortunately, not all the planned hauls could be performed every year and in 2018 the entire spring survey was cancelled due to ship unavailability. However, the remaining performed hauls were equally allocated among areas with different management regimes. The trawling protocols are as well the same for both surveys [72,87]: in accordance with the peak of *Nephrops* emergence [53,60], hauls (1 hour duration) were all conducted between 100 m and 270 m depth at sunset and sunrise using an experimental net (mesh size of 22 mm in the body and 12 mm in the cod end) and trawl sensors to calculate the swept area [72]. CPUE estimates for *Nephrops* for each haul were then calculated as total weight of the caught individuals divided by swept area (kg/km^2; hereafter referred to as biomass index) and number of caught individuals divided by swept area (N/km^2; hereafter referred to as density index) (S2 - S5 Figs) [72].

Due to the oceanographic peculiarities of the study area, CTD (Conductivity Temperature Depth) casts were also carried out during the surveys in order to capture spatio-temporal variations of environmental parameters at small scales [89,90]. Relevant oceanographic parameters (i.e. temperature, salinity and oxygen) measured as close as possible to the seabed (hereafter bottom data) where *Nephrops* lives were thus used as proxy to assess environmental variability over time and space. Therefore, to collect temperature and salinity data to be directly associated with each obtained CPUE value, CTD casts (by means of a *SBE19plusV2* probe) were performed as close as possible to the fishing location, before or after each haul [72]. The CTD was also equipped with a *SBE43* dissolved oxygen (concentration and saturation) sensor. A SBE5T pump ensures a constant flow over time inside the sensors

Model building

The effects of spatial and temporal variations of environmental parameters and fishery management measures on the CPUE indices were modeled by GAMs due to the high flexibility of the latter [31,91]. Preliminary analyses (not reported here) provided confidence that merging the spring and autumn time series increased the model accuracy owing to a bigger sample size and thus reducing overfitting, as shown also in Wisz et al. [92]. However, the two time series were previously filtered only for observations including all the information to be tested in the model. Indeed it was not possible to include all the available hauls in the model building process because some of these were lacking values for some of the environmental parameters. All hauls carried out within the 2012 spring survey were excluded due to lack of values for bottom dissolved oxygen saturation. For the rest of the spring survey time series, an average of 2.6 hauls for each year were removed (including the only two cases for which CPUE was equal to 0; S2 and S4 Figs); for the "autumn time series only one haul performed in 2017 was removed from the original dataset. A total of 56 (out of 89 available) hauls were selected within the spring time series from 2013 to 2019 (except for 2018), while in the autumn time series 36 (out of 37 available) hauls were selected.

The distribution of the response variables, i.e. biomass and density indices, appear not to be symmetrically distributed around the average with positively-skewed values and some outliers (e.g. highest values: 150 kg/km^2 for the biomass index and 17281 N/km^2 for the density index). Therefore, in the GAMs, a Gamma distribution with logit link function was assumed for both response variables; this distribution is one of the most used for environmental analysis [93], works well for positive-skewed data [94] and, owing to the logit link function, makes the model additive and therefore easier to interpret. To build the models, the covariates taken into account were: latitude (Y), longitude (X), depth (D), year (Yr), week of the year (week), time of the day (ToD), bottom temperature (BT), bottom salinity (Sal), bottom dissolved oxygen saturation (Oxy), and a factorial covariate accounting for restrictions on fishery (Fishery). The spatial scale is expressed by the "X" and "Y" covariates (in decimal degrees), which are the geographical coordinates referred to planned starting point of each performed haul, and by the “D” term (in meters) standing for the mean depth at which each haul was carried out. The spatial covariates are very important for *Nephrops* CPUE due to its sedentary behaviour [95]. The factor "Fishery" consists of 3 levels fitting with all the implemented managerial actions: fishery allowed (Y), limited fishery (L) (i.e. buffer zones regulation), and fishery ban (N). The temporal covariate “Yr”, expressed in numbers, was directly included as an interaction with the factor “Fishery” (using the “by” argument in the smooth functions) in order to model potentially different trends over years for each of the different fishery management levels. The inclusion of the intercept terms for categorical factor, as described in Wood [96], aims to increase the flexibility of the model fitting overall intercept differences between factor levels, and avoids artifacts; therefore, the “Fishery” factor was expressed both as intercept and as interaction with the covariate "Yr". Furthermore, the temporal scale was also expressed at different extents within the model equation (i.e. Year, week and ToD) in order to investigate the variability in catches between and within the seasons and times of day. Preliminary analyses (not presented here) carried out on the available dataset showed evidence that the “week” term, expressed as the number of the week within the year, is more suited (improves model performances) than other possible temporal scales (i.e. season or month during which the survey was conducted) to allow predictions by season (i.e. for the two survey seasons). The "ToD" covariate is instead a categorical factor representing the hauling time with two levels: sunset and sunrise (moments of the day at which correspond the peaks of maximum emergence from burrows of the species [55]). The terms accounting for the possible effects of environmental parameters recorded in correspondence of each trawl haul close to the seabed and already known to influence *Nephrops* life cycle and emergence behaviour (such as dissolved oxygen [40], salinity [50] and temperature [51], expressed as saturation percentage, practical salinity units, and *degree Celsius* respectively) were as well included in the models.

Before fitting the models on the data, in order to avoid multicollinearity, a covariate selection through Variance Inflation Factor (VIF) with backward selection was firstly carried out [97]. As a rule of thumb, the covariates with higher VIF should be excluded from the analysis one by one, until all the remaining variables have a VIF < 3 [98,99]. The analysis showed a high multicollinearity value (VIF > 3) only for the longitude covariate; hence the "X" term (mainly correlated with the latitude “Y”) was excluded from the considered models.

Model selection

According to the backward selection suggested by Zuur [97], a full model (hereafter referred to as initial model or “modINITIAL”) featuring all previously listed explanatory variables was formulated as follows for both density and biomass indices:

$$\mathrm{CPUE} = \beta^0 + s_1(Y) + s_2(D) + s_3(BT) + s_4(Oxy) + s_5(Sal) + s_6(week) +$$

$$s_7(Yr, by{=}Fishery) + f_1(Fishery) + f_2(ToD) + \varepsilon \tag{1}$$

Where CPUE corresponds to *Nephrops* biomass or density indices, β^0 is an overall intercept, s_i are penalized cubic regression splines, f_i indicate a categorical factor, the *by* symbol indicates that a spline function is separately estimated for each level of the factor, and ε is the error term. The models were all settled with Gamma error distribution with a logit link function and REstricted Maximum Likelihood (REML) as smoothing parameter estimation method [99]. Based on preliminary analyses (not reported here) a limit of 6 was selected as the maximum number of basis functions (k) due to the low number of data.

In the aim to investigate how including environmental variables and fisheries management measures at the same time in the model would perform and influence the quality of the CPUE derived estimations, three simplified models were as well tested and compared with the initial: one model excluding from “modINITIAL” both the effects of environmental variables and fishery management actions (“modNOEM”), one excluding the effect of the considered environmental variables (“modNOE”) and another excluding only the effect of fishery management actions (“modNOM”). The three simplified models were as well applied both to biomass and density; “modNOEM” was formulated as follows:

$$\mathrm{CPUE} = \beta^0 + s_1(Y) + s_2(D) + s_3(week) +$$

$$s_4(Yr) + f_2(ToD) + \varepsilon \tag{2}$$

“modNOE” was formulated as follows:

$$\mathrm{CPUE} = \beta^0 + s_1(Y) + s_2(D) + s_3(week) +$$

$$s_4(Yr, by{=}Fishery) + f_1(Fishery) + f_2(ToD) + \varepsilon \tag{3}$$

while “modNOM” was as follows:

$$\mathrm{CPUE} = \beta^0 + s_1(Y) + s_2(D) + s_3(BT) + s_4(Oxy) + s_5(Sal) +$$

$$s_6(week) + s_7(Yr) + f_1(ToD) + \varepsilon$$

(4)

In order to evaluate which of these four models was the best, intended as the most informative, a comparison among the Akaike Information Criterion (AIC) values was conducted for both indices. Indeed, the AIC accounts both for the goodness of fit and the complexity of the model allowing to select the best according to the lowest value [100]. As recommended by Zuur, the expected result was to select the model featuring all explanatory variables introduced according to the known biology and behaviour of the species (i.e. initial model), otherwise the simplest model (fewest covariates) would have been the choice [97]. A 10 k-fold cross validation analysis was thus performed according to [101]. Therefore, for both CPUE datasets (biomass and density indices) and for each of the four equations, 10 runs were carried out, using a random defined 90% of the dataset to train a model. This operation was repeated 10 times, each time excluding a different portion of the dataset (corresponding to 10%). Then the performance of each trained model was evaluated using AIC. The obtained 100 AIC values for each of the models ("modINITIAL", "modNOEM", "modNOE" and "modNOM") were assessed for homogeneity of the variance by the Levene's test and then (in case of homoscedasticity) analysed by means of a parametric one-way ANOVA with post-hoc Tukey test [102]. The ANOVA was conducted to evaluate if there was a significant difference within the means of the AIC values across the four tested models (levels of the factor) for both indices.

The model selected by means of the above described AIC comparison was then further refined excluding the non-significant terms. The significance of each covariate was verified through an evaluation of the 100 p-values obtained from the cross-validation for both indices; as a rule of thumb, if in 80% of cases the p-values was above 0.05, thus the covariate was retained in the equation. After that, in order to evaluate the model prediction performance by the Root Mean Squared Error (RMSE), a second 10 k-fold cross validation analysis repeated 10 times was again run for both initial and selected model. Once verified that the latter model, including only the significant covariates, was the most reliable to conduct predictions on both the CPUEs, this was further refined tuning it on the entire dataset. The adaptation of the models to the data was evaluated by computing the proportion of the null deviance explained (i.e. percentage of deviance explained) and the adjusted R^2 [94]. Furthermore, the deviance explained by each term of the final model was estimated from the model outputs according to Wood et al. [96]. The final model was meant to be used for indices predictions to be carried out on the whole area of interest (see paragraph below). All the statistical analyses were carried out using the statistical software R ver. 3.5.2 [103], the associated "mgcv" and "car" packages [94,104].

Indices predictions

In order to carry out, through the final model, predictions of the CPUE indices over the study area, a spatial grid of the Pomo/Jabuka Pits area, 2x2 nautical miles, was built by means of the Geographic Information System (GIS) Manifold® System Release 8 (http://www.georeference.org/doc/manifold.htm). Each cell is identified by geographic coordinates (Lat=Y, Lon=X) corresponding to its center; average depth values were as well assigned to each cell by means of a source layer (available from: [75]) and the GIS Spatial Overlay function. Furthermore, the grid was replicated for each survey/year combination according to their spatial domain (i.e. autumn surveys are conducted only in the western side; Fig 1). The fishery management measures in place (according to the regulations in force during the various survey periods) were as well assigned to each cell. In order to inform each cell of the grids, at survey (as a proxy of season) and yearly levels, with average bottom values for each considered environmental parameter (i.e. BT, Oxy and Sal), it was taken advantage of the direct availability of reliable data sets; the same values collected during each survey and previously used in association to each recorded CPUE value to build the GAMs, were thus interpolated using a kriging method by means of the Surfer software (Surfer® 11.6 from Golden Software, LLC: www.goldensoftware.com). The obtained layers were then superimposed on the grids by means of the GIS in order to assign average bottom values to each single grid cell (using again the Spatial Overlay function; S6 – S11 Figs). Notwithstanding the dissolved oxygen percentage was not measured in spring 2012, the values recorded in the same season of the following year (i.e. 2013) were assumed as proxy within this study; a similar assumption was made for spring 2018 (missing year in the time spring series) for which all environmental values were simulated using those recorded in spring 2019. In fact, to pursue the aims of this study it was decided not to include datasets derived from different sources that could possibly introduce different estimation bias.

All predictions were then carried out by cell, also considering a selected level of the "ToD" term (sunrise) to standardize for the daytime temporal domain while at the same time modelling differences in CPUE within seasons/years. Predictions obtained for each cell/survey/year combination by means of the final GAMs (see paragraph above), for both biomass and density indices, were then averaged (according to the spatial domain of each survey) to obtain new standardized indices that were then compared with the mean observed CPUE (average of hauls values).

Results

Model selection and fitting

The Levene's test indicated homogeneity of the variance for the AIC values obtained from all the tested models (“modINITIAL”, “modNOEM”, “modNOE”, “modNOM”), both for the biomass index (F

= 0.2296; df = 3.396; p-value = 0.8757) and density index (F = 1.0907; df = 3.396; p-value = 0.352). The results of the parametric one-way ANOVA showed a significant difference in the means of the AIC values (calculated on 100 values through cross-validation) across the different tested models (grouping variable) both for biomass index (F = 501.7; p-value = 2e-16) and density index (F = 176.1; p-value = 2e-16; S1 and S2 Tables). The Tukey post hoc test on the mean AIC showed significant differences for all the compared pairs of models both for biomass and density indices (S3 and S4 Tables). “modINITIAL” has the statistically lowest average AIC value than “modNOE”, “modNOM” and “modNOEM” (respectively not including environmental parameters, fishery management factors and both; S5 Table); “modINITIAL” may thus be considered the best model. Indeed “modINITIAL” resulted to be the most informative among the four tested GAMs, including at the same time the effect of environmental and fishery factors. The number of significant p-values obtained by the first cross validation analysis for all the covariates included in “modINITIAL” is shown in Table 1.

Table 1. Number of significant p-values for each term of “mod INITIAL". Number of significant p-values (< 0.05) out of 100 p-values in the 10 k-fold cross validation repeated 10 times for each covariate in the initial model for biomass and density indices.

Covariate	**Biomass index (kg/km^2)**	**Density index (N/km^2)**
Y	99	97
D	100	100
BT	18	9
Oxy	96	100
Sal	98	91
Week	0	0
ToD	100	100
FisheryL	95	99
FisheryN	89	57

Yr:FisheryY	100	100
Yr : FisheryL	0	1
Yr : FisheryN	100	99

The “BT” covariate was seldom significant in “modINITIAL” both for biomass and density indices (respectively 18 and 9 times out of 100), while the “week” covariate was never significant (0 out of 100). Accordingly, these two covariates (i.e. “BT” and “week”) were not included in the final models. Although the p-values obtained for the interaction between the covariate "Yr” and the factor "Fishery" were 0 and 1 (respectively for biomass and density indices) when the “Fishery” level was “L”, this term was retained in both the final models because, for the two other levels of the factor (“Y” and “N”), the p-values were 100% of the times significant. Therefore, the final model (“mod FINAL”) for both CPUE indices was formulated as follows:

$$CPUE = \beta^0 + s_1(Y) + s_2(D) + s_4(Oxy) + s_5(Sal) + s_7(Yr, by = Fishery) + f_1(Fishery) + f_2(ToD) + \varepsilon \quad (5)$$

The RMSE of the final model evaluated by running a second time the 10 k-fold cross validation analysis repeated 10 times resulted in lower averages than the initial model both for biomass and density indices (S6 Table); it was thus considered as the one with the best prediction performance. The final model (Eq. 5) included only significant explanatory terms; most of them were retained as single effects (i.e. Y, D, Oxy, Sal, Fishery, ToD) except for the interaction between "Yr" and "Fishery" factors. Tables 2 and 3 show the model parameters as tuned on the whole dataset. The final model explained 63.4% of the deviance (with an adjusted R^2 of 0.544) for the biomass index; while the explained deviance for the density index model was 66.9% (with an adjusted R^2 of 0.543). Percentages of deviance explained by each covariate of the final models both for biomass (kg/km^2) and density (N/km^2) indices are shown in the Supporting Information (S7 Table). No specific issue emerged from the analysis of the residuals: they showed homogeneity of variance and a mean around zero (S11 and S12 Figs).

Table 2. Summary of the outputs of the final GAM for the biomass index (kg/km^2). Explanatory variables included the single effect of factor Time of the Day (ToD) and Fishery, Year-Fishery interaction (Yr:Fishery) for each level of the factor (“Y” fishery allowed, “L” limited fishery, “N” fishery

ban), longitude (Y), depth (D), percentage of dissolved oxygen (Oxy) and salinity (Sal); "SE", standard error; "edf", estimated degrees of freedom; "df", degrees of freedom.

Parametric coefficients:	**Estimate**	**SE**	**t-value**	**Significance level**
Intercept	3.29	0.10	31.93	p<0.05
f(ToD)sunset	-0.39	0.10	-3.74	p<0.05
f(Fishery)L	0.65	0.22	2.99	p<0.05
f(Fishery)N	0.66	0.23	2.88	p<0.05
Smooth terms:	**edf**	**df**	**F**	
Yr : FisheryY	0.96	5	5.29	p<0.05
Yr : FisheryL	0.60	3	0.51	0.11
Yr : FisheryN	1.58	3	7.35	p<0.05
Y	0.90	5	1.88	p<0.05
D	3.88	5	16.15	p<0.05
Oxy	1.57	5	1.66	p<0.05
Sal	0.92	5	2.20	p<0.05

Table 3. Summary of the outputs of the final GAM for the density index (N/km^2). Explanatory variables included the single effect of factor Time of the Day (ToD) and Fishery, Year-Fishery interaction (Yr:Fishery) for each level of the factor ("Y" fishery allowed, "L" limited fishery, "N" fishery ban), longitude (Y), depth (D), percentage of dissolved oxygen (Oxy) and salinity (Sal); "SE", standard error; "edf", estimated degrees of freedom; "df", degrees of freedom.

Parametric coefficients:	**Estimate**	**SE**	**t-value**	**Significance level**
Intercept	7.61	0.11	72.43	p<0.05

f(ToD)sunset	-0.35	0.10	-3.39	p<0.05
f(Fishery)L	0.46	0.20	2.24	p<0.05
f(Fishery)N	0.72	0.20	3.64	p<0.05
Smooth terms:	**edf**	**df**	**F**	
Yr : FisheryY	1.71	5	9.34	p<0.05
Yr : FisheryL	0.44	4	0.20	0.169
Yr : FisheryN	0.94	4	3.62	p<0.05
Y	0.85	5	1.24	p<0.05
D	4.02	5	22.21	p<0.05
Oxy	2.05	5	3.31	p<0.05
Sal	0.92	5	2.26	p<0.05

Tables 2 and 3 also show the summary of the outputs of the final models for both the response variables. About the single categorical covariates, both tables show that the ToD term was significant and its effect was that average CPUE were lower at sunset than at sunrise (about 32% and 30% lower in case of biomass and density indices, respectively). Also the Fishery covariate had a significant impact and its effect was that with limited fishery (level "L") average CPUE were 92% and 58%, respectively for biomass and density indices, higher than when fishery was allowed; on the other hand, with no fishery (level "N") the average CPUE were 93% and 105%, respectively for biomass and density indices, higher than when fishery was allowed (Tables 2 and 3). The partial contribution of each continuous covariate for both biomass and density models are shown in Figs 2 and 3, respectively. The trend of Fishery interaction with the temporal variable (i.e. "Yr") (Figs 2 and 3, upper panels) showed a positive effect on catches when fishing effort was absent (level "N"), on the other hand a negative impact was highlighted in presence of an unmanaged fishing effort (level "Y"); no significant effect was detected with limited fishery (level "L"). As regard to the spatial terms, the spline of latitude (i.e. "Y") (Figs 2 and 3, middle left panel) had a slight influence on both models; the catch rates slightly decreased from the South to the North of the Pomo/Jabuka Pits area. The plots of both models indicated the "D" term (depth) as one of the covariates with the most evident impact (Figs 2 and 3) showing a positive effect up to about 175 m, then the CPUE indices decreased since almost 225 m after which they become constant (Figs 2 and 3, central panel). The effect of "Oxy" for both CPUE indices (Figs 2 and 3, middle right panel) indicated a slightly negative impact on catch rates until almost 85% after which it becomes strongly positive, leading to a consequent increase in the CPUE indices. The effect of the "Sal" term (Figs 2 and 3, bottom left panel) showed a negative impact on catch rates when salinity values increase; these effects were observed for both models.

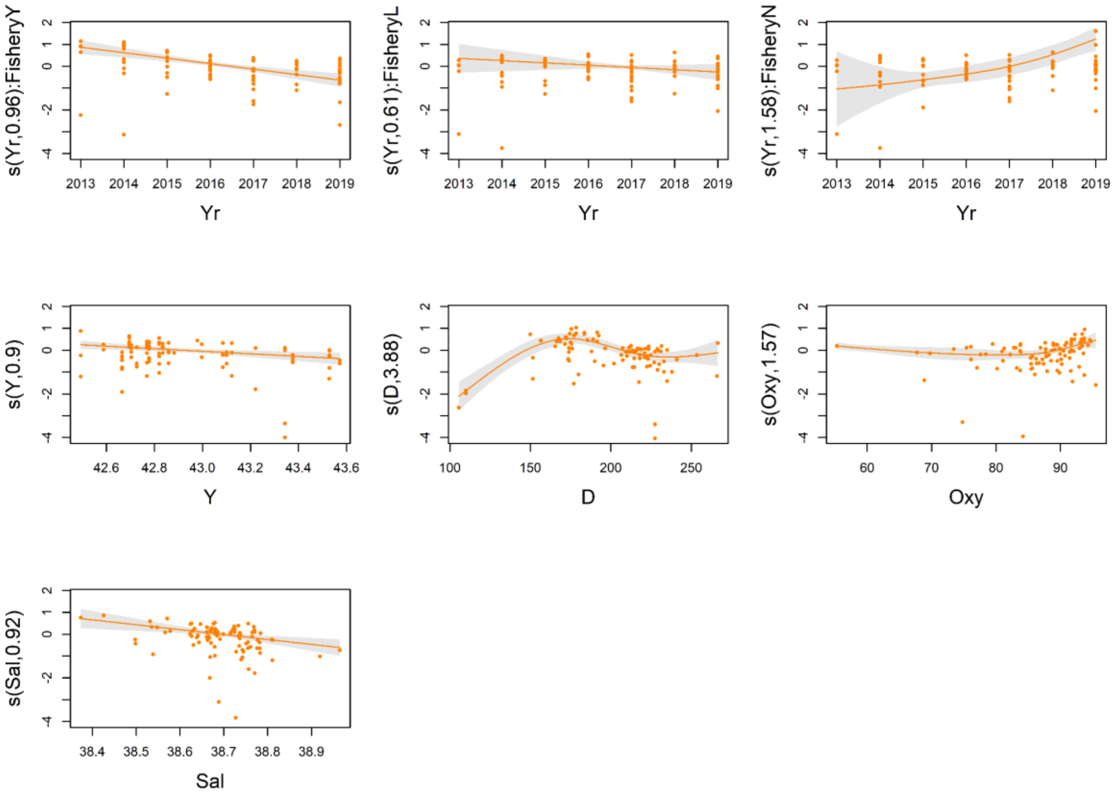

Fig 2. Partial effects plots of GAM on *Nephrops* CPUE (kg/km^2). Partial effects (y axis) of spatial (Y, D), environmental (Oxy, Sal), and fishery management variables (Yr:Fishery) selected for the final GAM. Grey shaded regions indicate the 95% confidence intervals, dots are the residuals.

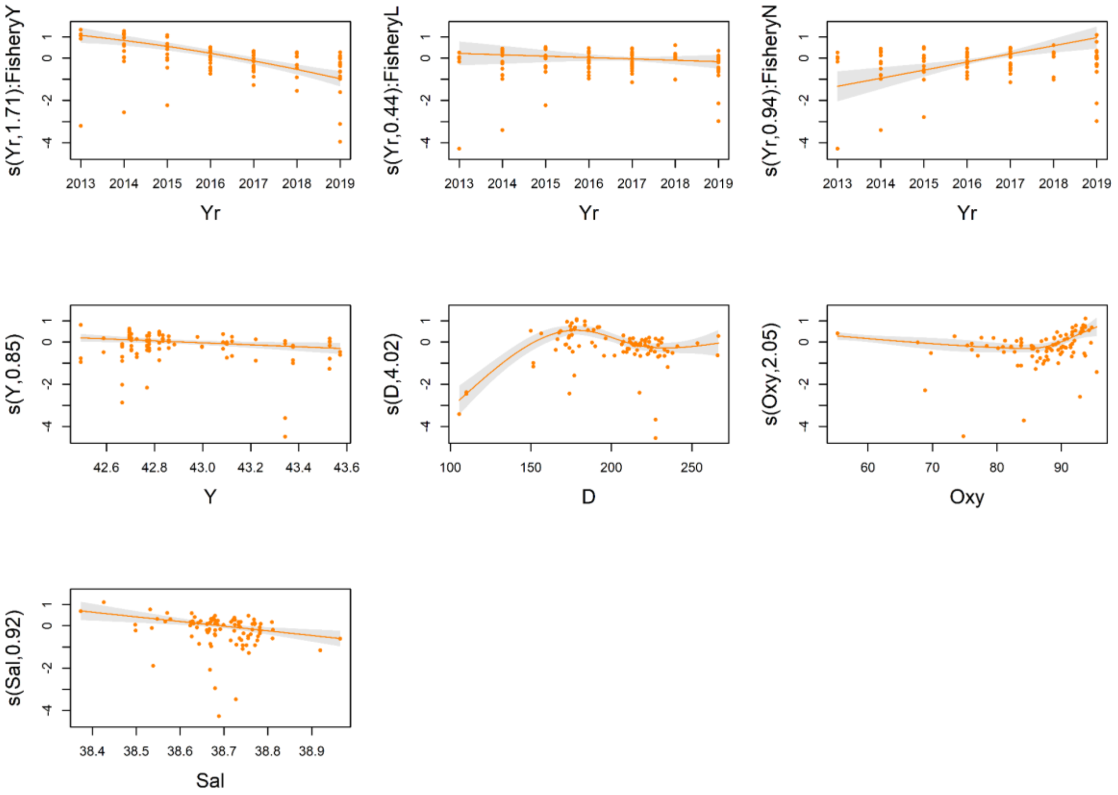

Fig 3. Partial effects plots of GAM on *Nephrops* CPUE (N/km^2). Partial effects (y axis) of spatial (Y, D), environmental (Oxy, Sal), and fishery management variables (Yr:Fishery) selected for the final GAM. Grey shaded regions indicate the 95% confidence intervals, dots are residuals.

Predictive distribution maps

Figs 4 - 7 show the annual predictions of *Nephrops* biomass and density CPUEs per cell, within the spatial domains previously defined by means of grids (in the Pomo/Jabuka Pits study area), for the spring and autumn time series.

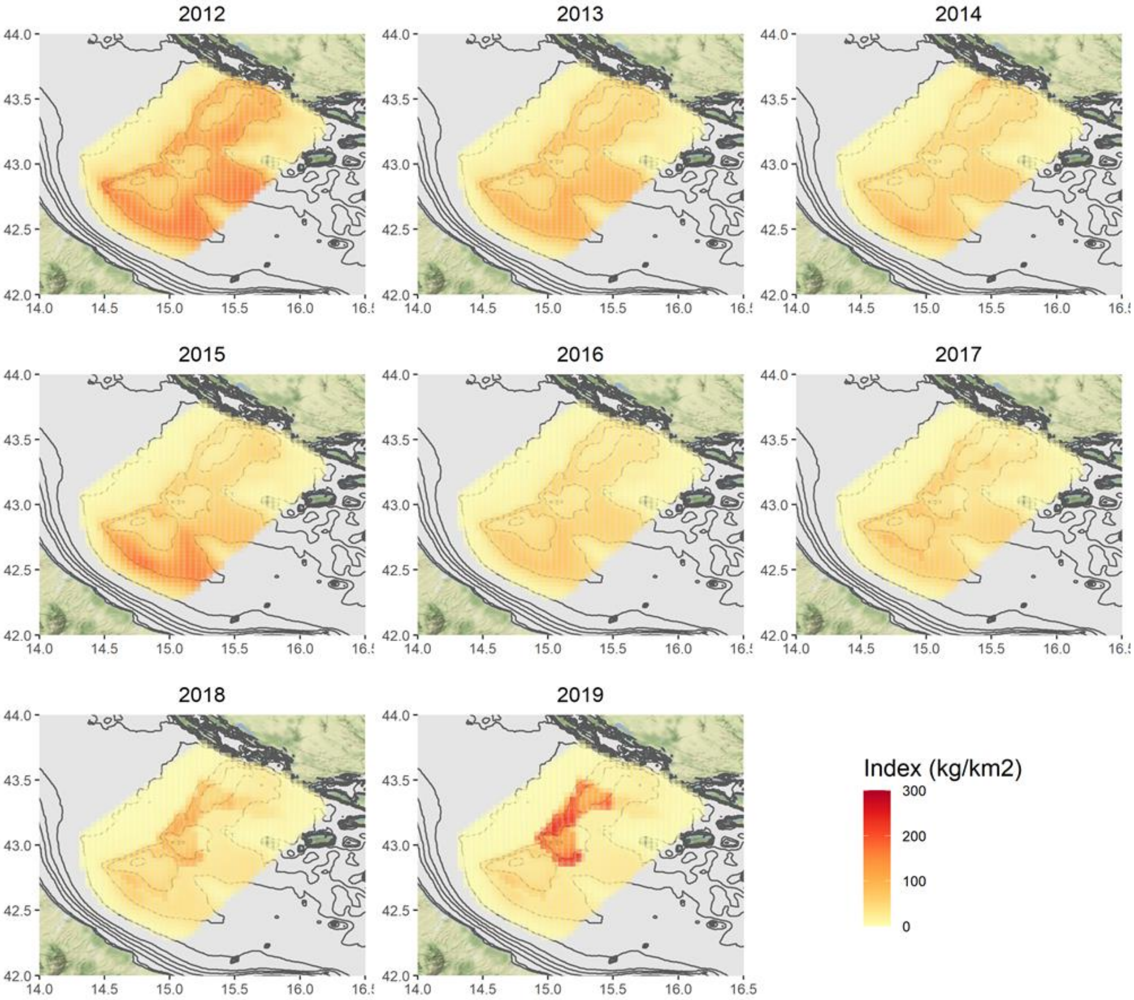

Fig 4. Predicted spatial distributions of *Nephrops* biomass index (kg/km^2) for the spring time series. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.

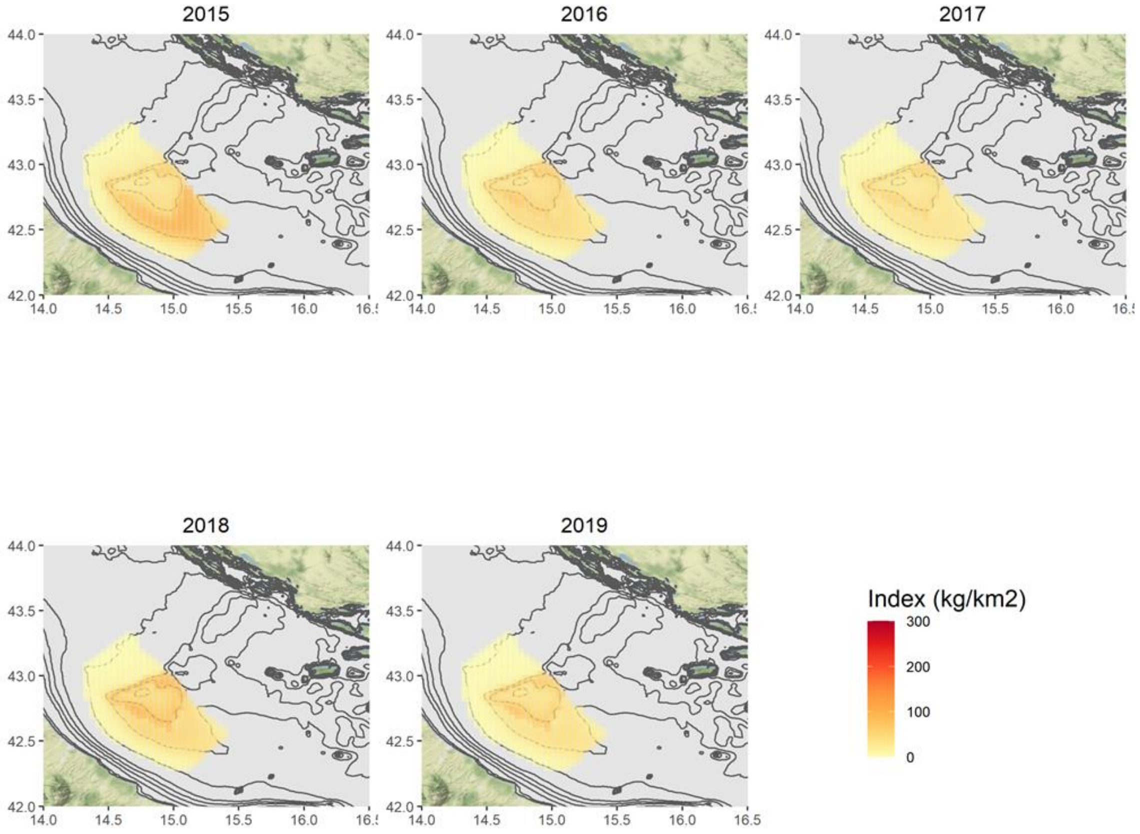

Fig 5. Predicted spatial distributions of *Nephrops* biomass index (kg/km^2) for the autumn time series. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.

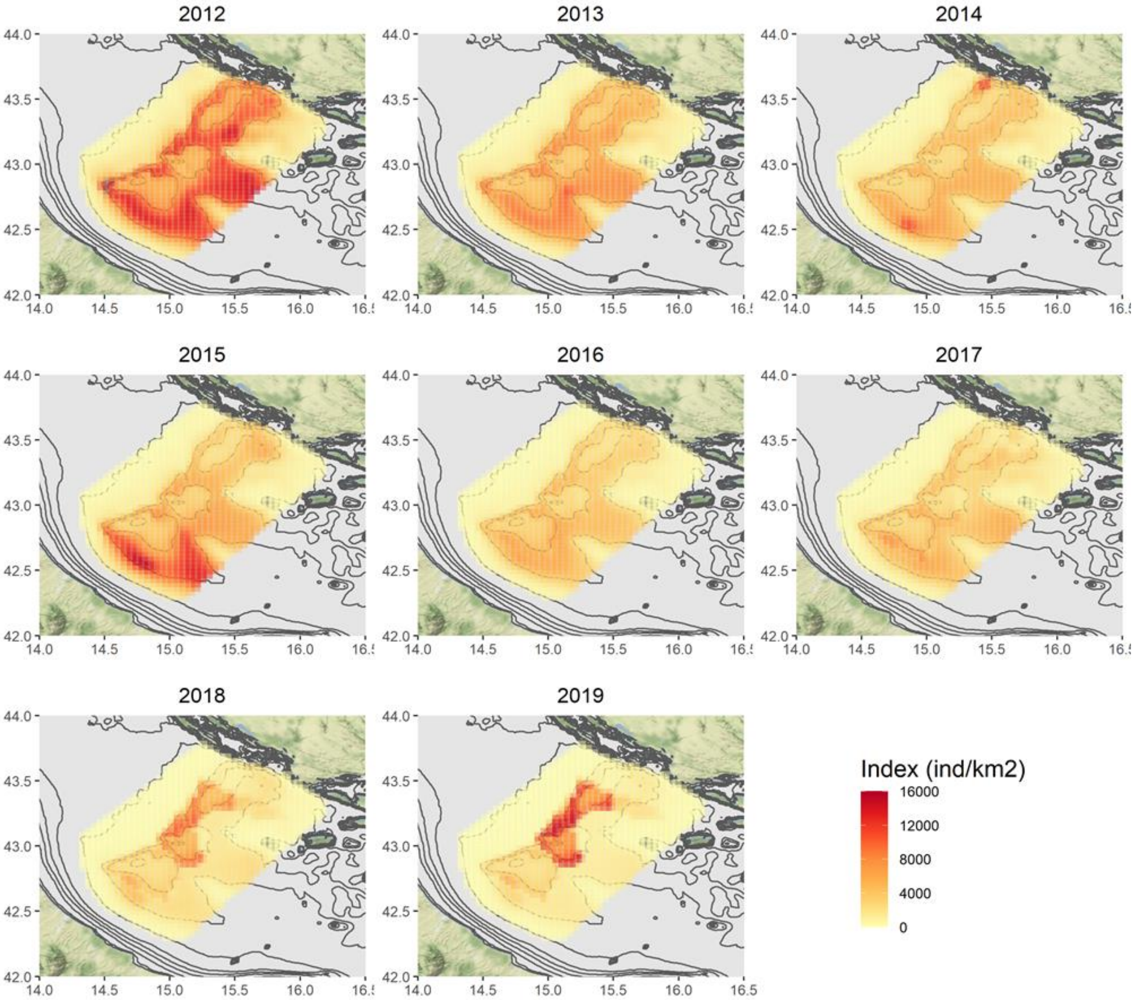

Fig 6. Predicted spatial distributions of *Nephrops* density index (N/km^2) for the spring time series. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.

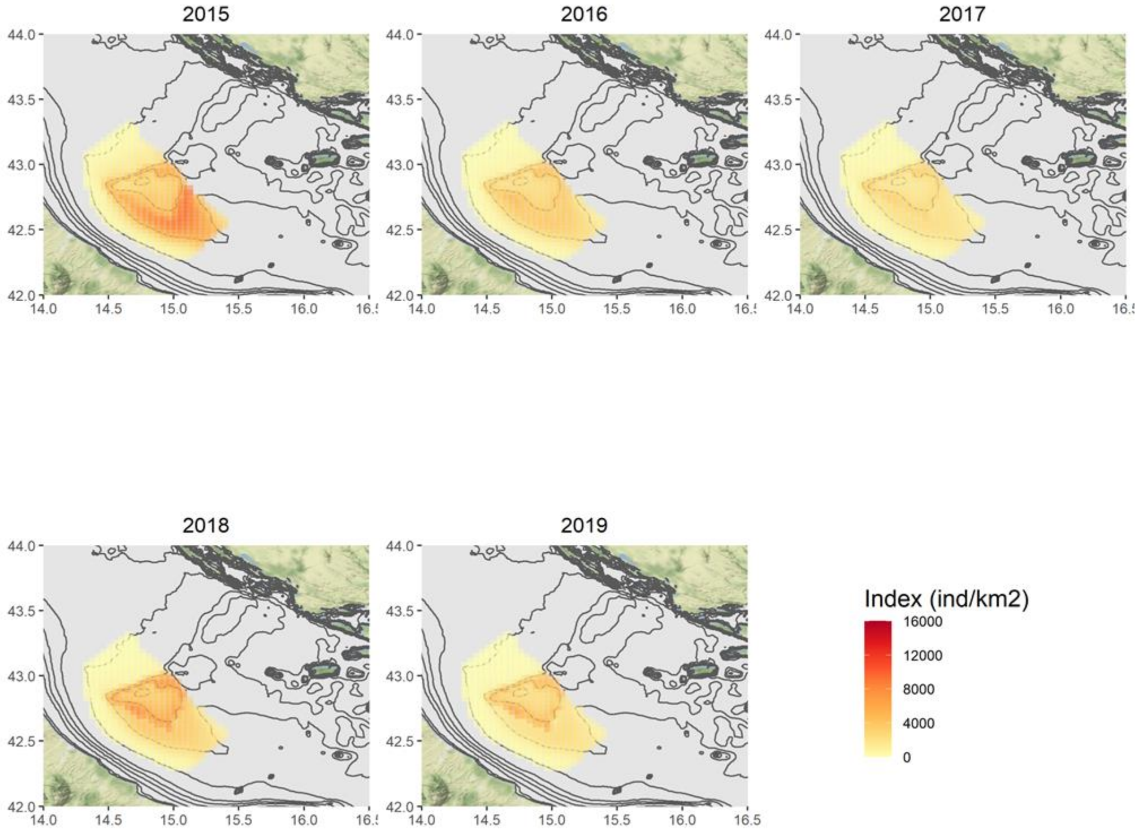

Fig 7. Predicted spatial distributions of *Nephrops* density index (N/km^2) for the autumn time series. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.

Maps of the related standard errors (per cell) are available in the Supplementary Material (S14 – S17 Figs). Overall, the predicted distribution in space and time of the biomass CPUE (kg/km^2) seems to follow the same patterns of the density one (N/km^2), even if the numerical predictions are of course different. The highest estimates for the spring time series in the period 2012-2017, were obtained for the cells localised around the Pomo/Jabuka Pits at depths from about 150 m to 200 m and in the south-eastern part of the study area. By contrast the distribution of *Nephrops* indices in the last two modelled years (i.e. 2018 and 2019) was almost pooled within the boundaries of the fishing ban zone (i.e. zone "A"). For the autumn time series, the highest predictions were centered in the southern part of the study area as well, with the exception of 2017, 2018 and 2019 for which the highest predicted catches were within the boundaries of the FRA zone "B", from about 180 m to 200 m of depth. All the modelled indices showed very low or even no predicted values for the cells in the north-west zone of the study area.

Standardized CPUE values

Fig 8 shows a comparison between mean observed CPUEs (average of hauls values per year and survey) and mean values obtained by means of the final GAMs (average of values obtained for each cell/survey/year combination), for both biomass and density indices.

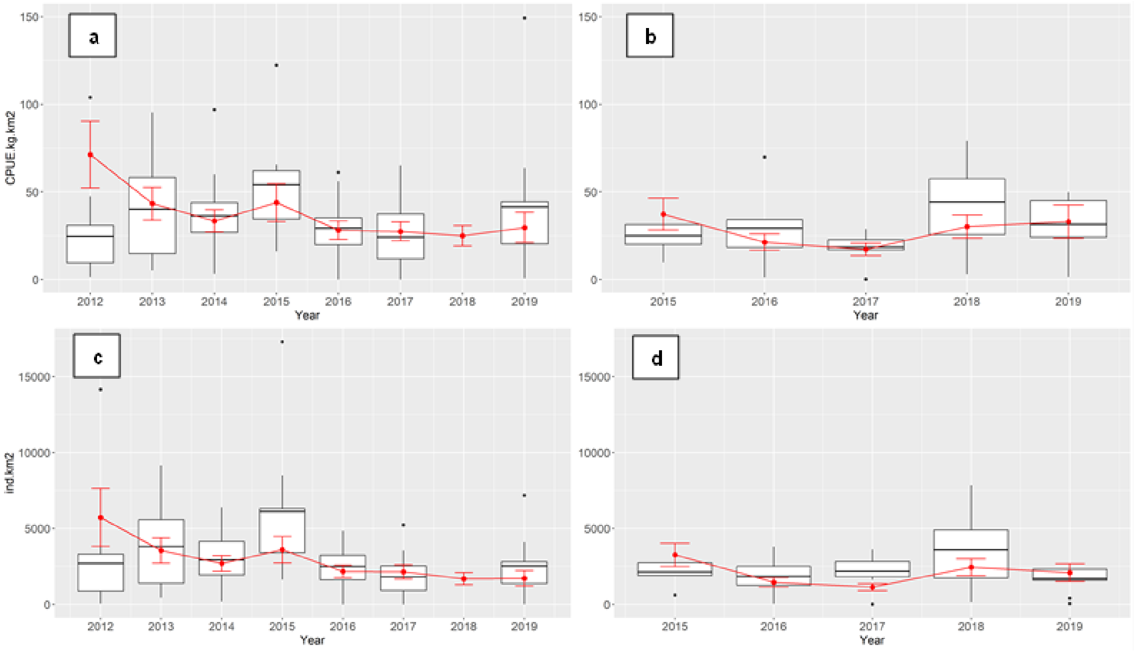

Fig 8. CPUE indices. Boxplots for the unstandardized time series over years represented with mean values (horizontal black lines), third and first quartiles (top and bottom vertical black lines, respectively) and outliers (black dots) plotted against the mean of predicted annual CPUE obtained for each cell of the grid (red dots) with standard errors (red lines). The spring time series is on the left (*a* and *c* panels), the autumn time series is on the right (*b* and *d* panels). Biomass index is represented in the upper panels (*a* and *b* panels), while density index in the lower panels (*c* and *d* panels).

For the biomass models, the maximum difference between observed and predicted mean values in the spring survey time series was found in 2012 with an increment of about 65% from 24.6 kg/km^2 to 71.3 (± 19) kg/km^2 (Fig. 8, panels a); in the autumn survey time series an important difference was found in 2015 with an increment of about 33% from 24.9 kg/km^2 to 37.33 (± 9.1) kg/km^2 (Fig. 8, panels b). Apart from these two exceptions, the mean predicted values for the biomass index were always within the interquartile range (i.e. middle 50% of the observed data) (Fig. 8, panels a and b). For the density models, the highest discrepancy in the spring survey time series was as well detected in 2012, with a huge increment from about 2704 N/km^2 to 5723.27 (± 1910.1) N/km^2 (Fig. 8, panel c). Differently from the biomass models, for density indices in the autumn survey time series the maximum differences of about 50% were found in 2015 and 2017, with an increment from 2129 N/km^2 to 3263.3 (± 773.2) N/km^2 and a decrement from 2188 N/km^2 to 1134.9 (± 237.3) N/km^2 respectively (Fig. 8, panel d). Excluding these cases, all the mean predicted values were always within the interquartile range of the observed data (Fig. 8, panels c and d). With the exception of 2018, the mean values of the predicted CPUE for the spring survey time series are higher than those for the autumn survey time series (Fig. 8). A concordance between the observed CPUE trends and the predicted ones was confirmed for the majority of the years within both time series. The mean predicted spring CPUE trends showed the highest peaks for both abundance and density indices in 2012 and 2015, together with the highest mean standard errors, and a slight increase of estimates in 2019 (Figs. 8a and 8c). For the mean predicted autumn CPUE trends, the highest peaks and standard errors for both indices were detected in 2015 and in 2018 (Figs. 8b and 8d).

Discussion

In this study, GAMs were applied to produce specific standardized *Nephrops* indices accounting for both the effects of environmental variables and fishery management actions in place within the Pomo/Jabuka Pits (central Adriatic Sea). The raw CPUE time series treated in this work were never included in an official stock assessment before, thus the information here provided could also be relevant to feed future robust population dynamics models for this species. Standardization was carried out on both available indices (i.e. kg/km^2 and N/km^2) to investigate possible discrepancies over time and space between the two trends. In fact, for species such as *Nephrops* there could be possible differences due to the fact that density may be size dependent [106]. Furthermore, some stock assessment tools can be fed alternatively by both biomass and density indices (e.g. Stock Synthesis 3 and C++ algorithmic stock assessment laboratory [107,108]).

Filling the gaps of a time series through standardization with GAMs

Poor weather conditions, limited shiptime, unavailability of vessels, equipment failures and other occasional issues could lead to shortcomings due to data unequally distributed in space and time along the survey time series [88]. It is well known that in case of fishery-dependent data, sampling strategies inadequate to the extension of the study area may result in ostensibly stable catch rates (i.e. hyperstability) or in not reliable trends changes (e.g. hyperdepletion) [109]. In case of fishery-independent surveys, spatial stratification could be adopted to minimize such bias in the abundance estimates so long as each stratum has an approximately homogeneous density [110]. Moreover, when the study area is not uniformly surveyed due to biased sampling design (e.g. an occasional lack of data in some planned sampling locations) standardization of catch rates is recommended to remove most of the variability not directly attributable to changes in abundance [30]. Distribution models including environmental effects should thus be preferred to predict species distributions and abundances at local scales [111,112]. However, when dealing with gaps in the spatiotemporal distribution of marine resources GAMs are more convenient than GLMs because they can easily incorporate the nonlinear responses of catches to geographic factors by smoothing rather than stratifying [32,33]. On the other hand, GAMs are likely to cause overfitting, especially with small sample sizes, because they allow the use of several fixed effects in nonlinear smoothing functions, which often reduce the predictive ability [113]. In this study, GAMs were selected as a suitable tool for filling the gaps inside the time series and at the same time explain the influence of environmental processes on the species distribution. In the future, it would be interesting to compare this standardization modelling approach with other spatio-temporal models which also include autoregressive processes [35,114].

Spatio-temporal distribution of estimated CPUE

Given that the seasonal emergence rhythmicity of *Nephrops* is historically demonstrated in the Adriatic Sea [57], an intra-annual temporal term (i.e. “week”) was thus included in the model building process: unfortunately no significant differences between catch rates from the 2 surveys were found. Moreover, the available data set was probably too little extended over the years to allow the detection of intra-annual CPUE variability. However the informed grids allowed to predict seasonal indices relying on the availability of environmental data for spring and autumn. Seasonal differences in emergence patterns were addressed for different stock of a similar species, *Metanephrops challengeri* (Balss, 1914), by using assessment models able to include seasonal abundance indices [115]. Therefore, it would be advisable to develop in the near future similar solutions also for Mediterranean *Nephrops* stock, as suggested by Aguzzi et al. [51]. Hence, it would be important to maintain the consistency of the 2 *Nephrops* targeted seasonal time series in order to improve the understanding of spatio-temporal dynamics and to provide increasingly accurate stock assessment inputs. Furthermore, when dealing with small data sets it is not possible to select a large number of predictors, otherwise this could lead to an increase in the uncertainties [96]. Hence, in the model building process, only the main effects for all the covariates were tested with the exception of the managerial factor which was expressed both as intercept and as interaction with the covariate "Yr". As recommended by Wood, an intercept term needs to be included when using ordered factors because smooths with factors are affected by the centering constraints [96]. As might be expected, both GAMs estimate increasing catch rates when fishery is absent (level "N") and decreasing ones when fishery is allowed (level "Y"); on the other hand it is interesting to note that in presence of partial fishery restriction measures (level "L") the catches do not increase but remain stable. This could be explained by: (i) a time series not long enough to observe significant changes in the abundance; (ii) a too little reduction of fishing effort in order to observe an increase in the catch rates. However, following the implemented regulations and their changes in time and space, the levels of the "Fishery" factor for fishing ban ("N") or limitations ("L") were fixed in the grid from autumn 2015 forward; given the actual explanatory power of "Fishery" factor, it is therefore plausible that since then the managerial actions influenced the distribution of the estimated indices. This is also supported by some recent studies showing a clear alteration of size and density in crustacean species when a no-take-zone or fishery ban periods are established [116–118]. Indeed, considering the predictions for the cells set at level "N" for the Fishery factor from 2018 onwards (corresponding to the fishery ban area "A") an increment in CPUEs was found. Furthermore, the highest values of catches are mainly concentrated in the cells positioned around the Pits at a bathymetric range from about 180 m to 200 m. Actually, the depth variable proved to be one of the main factors influencing the spatial distribution of *Nephrops*. Both models presented here show that there is a positive effect on the catch rates for the above mentioned bathymetric range, suggesting the presence of higher values of density and biomass indices along the borders of the Pits rather than within the Pits (< 200 m).

Overall, a slightly positive spatial gradient from the North to the South of the Pomo/Jabuka Pits area is supported by the spline of the “Y” term. Such an effect can be easily observed in the predictions for the period before the implementation of the first management measure (i.e. spring 2015) for which the spatial distribution of the indices within the maps were mostly centered in the south-east zone of Pomo/Jabuka Pits. A pretty similar distribution of Landings Per Unit of Effort (LPUEs) was previously estimated by a model developed by Russo et al. [56] which estimates *Nephrops* landings per fishing ground combining landings per harbor and fishing vessels routes from VMS (Vessel Monitoring Systems [119]). Besides, a displacement model by Bastardie et al. showed a possible redistribution of the fishing effort toward surrounding areas in case of establishment of a fishing ban within the Pomo Pits [28]. This could therefore support the reduction of catches in the southern part of the prediction area (not subject to fishery limitations) after the implementation of the management measures. The agreement with the local (i.e. Pomo/Jabuka Pits) spatio-temporal distribution of *Nephrops* is an evidence of the great spatial accuracy of the models presented here.

Roles of environmental variables in the standardization models

Changes in biomass and density over time and space are the results of interactions among individuals and between individuals and their environment; hence, understanding of adaptive behaviours in response to changes in the environment helps to explain the complexity in spatiotemporal distribution [109]. One of the main processes influencing the environmental conditions in the Pomo/Jabuka Pits is the periodical, though occasional, water mass renewal caused by dense water formation over the northern Adriatic shelf [73,120,121]. The Middle Adriatic Deep

Water (MAdDW), that resides throughout the year in the bottom layer of the Pomo/Jabuka Pits, is periodically renewed by Northern Adriatic Densev Water (NAdDW) at one to three years intervals but occasionally at longer intervals [73,120], leading to a density and oxygen increase and to a temperature and nutrient decrease [122]. Such renewals have an effect on the local biodiversity and on the trophic status of benthic communities [123].

In the model selection section, the initial model was built and then compared to other three models derived firstly by removing both the environmental covariates and the terms related to the fishery management actions, secondly by removing only the environmental covariates and finally by removing only the fishery management terms. The aim was to statistically understand if the environmental and fishery covariates could effectively improve the model performances. The results of statistical analysis provided evidence that the model including both environmental and fishery covariates perform better than the other three. In both final models, a positive impact on catch rates was estimated for dissolved oxygen values greater than 85%. An exceptional dense water formation was reported during winter 2012 in the Central Adriatic Sea [124,125]. An average bottom dissolved oxygen saturation of 91% was recorded in late March 2012 in a transect carried out in the western side of the Pomo/Jabuka Pits area, while an average of 81% was recorded for the same stations in April 2013 (CNR IRBIM unpublished data); thus the use of the 2013 dissolved oxygen values as a proxy for 2012 could probably have led to underestimation of the modeled indices in 2012 if not coupled with other explanatory environmental variables (i.e. salinity; see below). On the other hand, relatively high catches were estimated also for dissolved oxygen values lower than 60%. Laboratory studies show that low percentages of dissolved oxygen saturation within the substratum may force *Nephrops* individuals out of their burrows in an attempt to ventilate on the bottom surface [126]. In the Pomo/Jabuka Pits the bottom oxygen depletion caused by local "ageing" consequent on reduced ventilation could even lead to sediment hypoxia (less than 40% [122]). Historically, extraordinary hypoxic conditions in the Central Adriatic Sea associated with a sharp decline in both *Nephrops* landings and experimental catches were reported during the 1980's [127]. Such a stressful environmental condition could influence the catchability and the natural mortality of this species [40,128,129] even affecting the strength of recruitment [130]. Similarly, eutrophication events affecting the Kattegat and Skaggerak fishery in the mid-1980s led to a steep decrease of catch rates, as a consequence of high mortality [131,132]. No values attributable to hypoxic conditions were recorded along the time series presented here. However, very low levels of dissolved oxygen saturation were recorded in the area before the exceptional dense water formation in winter 2012 (e.g. 55-52% in 2010 and about 47% in 2011; CNR IRBIM unpublished data). Raw catch rates showed an increase of mean indices from 2012 to 2013, by contrast the model estimated a decreasing trend while maintaining high standard errors. Actually, the discordance between mean observed CPUE and the averaged prediction in 2012 for both indices could potentially also be influenced by the low level of oxygen saturation in the previous years which could have already reduced the abundance of *Nephrops*, similarly to what happened during 80's [127]. Given the fact that key environmental stressors could affect *Nephrops* at different life stages [133], future models should also be tested for the dissolved oxygen saturation recorded in previous years in correspondence with recruitment times (in Adriatic hatching happens yearly in late winter [40]), as suggested for sea surface temperature and pink shrimp (*Parapenaeus longirostris*) in Colloca et al. [134].

Nevertheless oxygen is not the only environmental parameter that could affect *Nephrops* catch rates; salinity stress conditions as hypercapnia and salinity fluctuations may as well alter abundance of recruits [135]. However, literature on salinity influence on *Nephrops* is poor and mainly focusing on survival studies, generally showing an increase in mortality in low salinity ranges (around almost 15 in Harris and Ulmestrand [50]; and from about 24 to 29 in Fox et al [136]). Within this study, in both models slight variations in salinity ranges resulted in relevant impacts on CPUE values, despite the measured range being really narrow. Both in spring 2012 and autumn 2015 (the periods in which the maximum CPUE predicted mean values were obtained for the two surveys) salinity showed generally lower estimates for the whole study area extent, if compared with those related to the respective following year/season combinations (S6 and S7 Figs), suggesting that such high estimates in the prediction could be influenced by these low salinity concentrations. However, the reasons behind these discrepancies in predictions, associated with high standard errors, could also be due to an insufficient amount of collected data, in particular for 2012. On the other hand, a negative impact of high salinity levels as the one observed within this study has never been reported in literature, thus further studies on salinity variation effects on *Nephrops* populations are suggested. As stated before, in this work it was not possible to test more than one interaction at a time; it is thus recommended that future studies will also take into account interactions between different environmental variables during the model building process. Further model developments should also include and investigate the role of other parameters known to potentially affect the CPUE of *Nephrops* such as sediment composition, tide level and the lunar cycle [45,48,137].

The importance of targeted sampling protocol for *Nephrops*

A common objective of population dynamics models is to employ observed CPUE indices to estimate the population abundance through time [109]. According to Cook [138], data collected by means of trawl surveys are a more accurate source of information for estimating stock abundance than observations from commercial landings. Despite that, owing to the temporal variation in *Nephrops* emergence behaviour, catch data from all survey activities may not be representative of the real population [55,85]. For example, the MEDiterranean International Trawl Survey (MEDITS [139]) which is the main index of abundance available for Mediterranean demersal resources, is not properly designed to catch *Nephrops* (e.g. hauls start one hour after dawn and stop one hour before dusk every day), indeed its estimates are also affected by its rate of emergence as for commercial trawl fishery [140,141]. In order to overcome these kinds of issues, in several European countries a specific methodology (namely UWTV) based on the use of a camera system for the detection of burrows numbers and derivation of density indexes was developed [142]. In trawl surveys to reach an adequate level of proportionality a representative portion of the population have to be sampled and the efficiency of the net have to be experimentally measured [143]. Both conditions are easily achievable during a fishery-independent survey thanks to a well-known and standard procedure [88,144]. It is the case of the 2 Adriatic trawl surveys reported here which were specifically designed to target *Nephrops*; indeed the trawl hauls are all conducted at the peak of emergence of the species (i.e. sunset and sunrise [55]) and the experimental net was designed to catch a representative portion of the population including juvenile individuals [72,87]. The reliability of the collected data is also proved by the almost total absence of zeros in the time series; such a condition is rarely confirmed in other demersal trawl surveys within the Adriatic Sea (e.g. MEDITS survey [145]). Furthermore, the presence from 2015 of another *Nephrops* targeted survey carried out during autumn season (i.e. “ScamPo”; [72]) allowed to collect information about population structure at a finer timescale. Such a level of information is an important tool for stock assessment purposes as it allows to consider the different availability of *Nephrops* sexes during the year [40,72,146] improving the input quality of population dynamics models.

Conclusions

The overarching goals of this study were to analyze how drivers such as environmental changes over time and alterations of fishing effort influence CPUE estimates and to use these outcomes to standardize the abundance indices from fishery-independent trawl surveys. The *Nephrops* subpopulation inhabiting the Pomo/Jabuka Pits was selected as a case study mainly due to behavioural characteristics of this species that may affect catches, the oceanographic peculiarities of the study area and the implementation and subsequent change in time and space of different fisheries management measures. The availability of trawl survey data collected along with environmental parameters allows to include in the model environmental effects directly related to the capture event. Among the investigated covariates the most explanatory ones resulted to be fishery and depth, while dissolved oxygen and salinity showed a relevant effect on catch rates. The standardization of CPUE from the targeted surveys allows predicting reliable indices for a sedentary species with a high variability in catch rate as *Nephrops*. In addition, through the use of GAMs, it was possible to fill the gaps within the historical time series, thus being able to provide a biomass or density index for all the considered time frame. Indeed, an informed grid was built in order to predict the indices at different spatial scales. The standardization for the two different time series led to estimate CPUE indices for each of the two seasons (i.e. spring and autumn) allowing to model the differences in the availability of *Nephrops* during the solar year. This is of relevant importance from the management point of view and, in future analyses, this standardized CPUE might improve the evaluation of the status of the resource at different temporal and spatial scales. Moreover, the achieved knowledge on the impact of fisheries and some environmental factors on *Nephrops* communities and their spatio-temporal dynamics in Pomo/Jabuka Pits could be of extreme relevance while developing an ecosystem approach to fishery management.

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Supporting information

S1 Table. Parametric one-way ANOVA for abundance models (kg/km^2). Analysis of the variance of AIC values across the four tested models for abundance. Hence, “model” is the factor, “Df” is the number of degree of freedom, “Sum Sq” represent the sums of squares (i.e. the distances of each point to the mean), “Mean Sq” is the mean of squares, “F” is the ratio of the sum of squares, “Pr(>F)” is the combination of F-statistic with the degrees of freedom. Asterisk (*) refers to the level of significance (>0.05).

	Df	Sum Sq	Mean Sq	F	Pr(>F)
model	3	105151	35050	501.7	<2e-16 *
Residuals	396	27664	70		

S2 Table. Parametric one-way ANOVA for density models (N/km^2). Analysis of the variance of AIC values across the four tested models for density. Hence, “model” is the factor “Df” is the number of degree of freedom, “Sum Sq” represent the sums of squares (i.e. the distances of each point to the mean), “Mean Sq” is the mean of squares, “F” is the ratio of the sum of squares, “Pr(>F)” is the combination of F-statistic with the degrees of freedom. Asterisk (*) refers to the level of significance (>0.05).

	Df	Sum Sq	Mean Sq	F	Pr(>F)
model	3	82820	27607	176.1	<2e-16 *
Residuals	396	62094	157		

S3 Table. Tukey multiple comparisons among AIC values of “modINITIAL”, “modNOEM”, “modNOM” and “modNOE” for abundance models (kg/km^2). Tukey post-hoc test (95% family-wise confidence level) conducted on averaged AIC values calculated from the 10 k-fold cross validation repeated 10 times for each combination of the four tested models (“modINITIAL”, “modNOEM”, “modNOE”, “modNOM”) for biomass index. “diff” indicates the difference between values, “lwr” is the lower bound of the confidence interval while “upr” is the upper bound, and “p adj” is the adjusted p-value.

	diff	lwr	upr	P adj
modNOEM - modINITIAL	-37.147301	-40.1968950	-34.097707	0.0000000
modNOEM - modNOM	-6.463387	-9.5129805	-3.413793	0.0000005
modNOEM - modNOE	-33.290466	-36.3400595	-30.240872	0.0000000
modNOM - modINITIAL	30.683914	27.6343206	33.733508	0.0000000
modNOE - modINITIAL	3.856835	0.8072416	6.906429	0.0065551
modNOE - modNOM	-26.827079	-29.8766729	-23.777485	0.0000000

S4 Table. Tukey multiple comparisons among AIC values of “modINITIAL”, “modNOEM”, “modNOM” and “modNOE” for density models (N/km^2). Tukey post-hoc test (95% family-wise confidence level) conducted on averaged AIC values calculated from the 10 k-fold cross validation repeated 10 times for each combination of the four tested models (“modINITIAL”, “modNOEM”,“modNOE”, “modNOM”) for density index. “diff” indicates the difference between values, “lwr” is the lower bound of the confidence interval while “upr” is the upper bound, and “p adj” is the adjusted p-value.

	diff	lwr	upr	p adj
modNOEM - modINITIAL	-36.925820	-41.494659	-32.35698	0.00e+00

modNOEM - modNOM	-7.769049	-12.337888	-3.20021	8.67e-05
modNOEM - modNOE	-24.573393	-29.142232	-20.00455	0.00e+00
modNOM - modINITIAL	29.156771	24.587932	33.72561	0.0000000
modNOE - modINITIAL	12.352427	7.783588	16.92127	0.0000000
modNOE - modNOM	-16.804344	-21.373183	-12.23551	0.0000000

S5 Table. Mean AIC of “mod INITIAL”, “modNOEM”, “mod NOM” and “mod NOE” for both biomass (kg/km^2) and density (N/km^2) indices. Mean AIC with standard deviation out of 100 AIC values from the first 10 k-fold cross validation repeated 10 times for each of the three four models (“modINITIAL”, “modNOEM”, “modNOE” and “modNOM”) for both biomass and density indices.

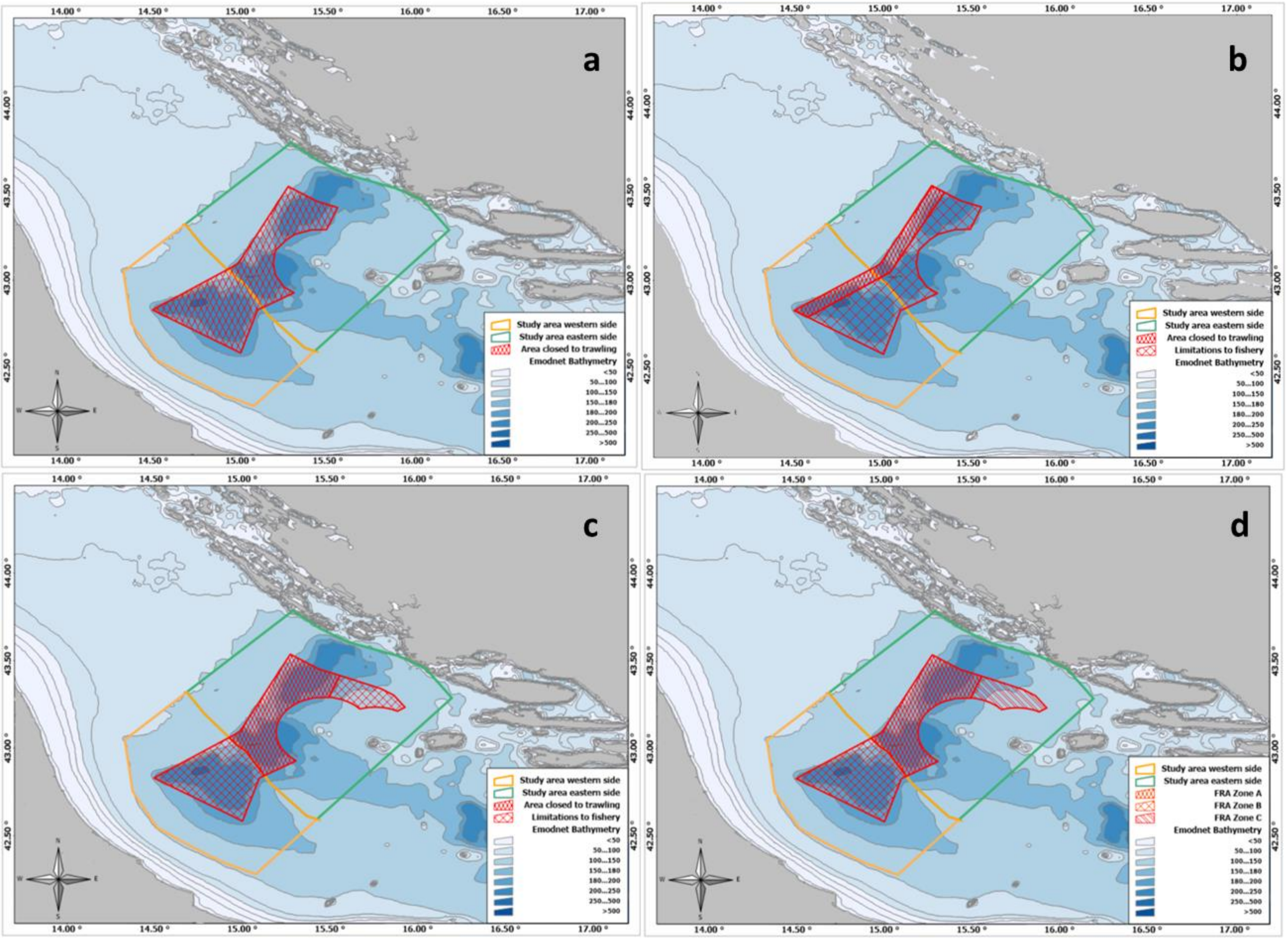
	Biomass (kg/km^2)	Density (N/km^2)
modINITIAL	677.485 ± 8.055	1407.745 ± 12.051
modNOEM	714.633 ± 8.267	1444.671 ± 12.668
modNOM	708.169 ± 8.586	1436.902 ± 11.663
modNOE	681.342 ± 8.515	1420.097 ± 13.619

S6 Table. Mean RMSE of “mod INITIAL” and “mod FINAL” for both biomass (kg/km^2) and density (N/km^2) indices. Mean RMSE with standard deviation out of 100 RMSE values from the second 10 k-fold cross validation repeated 10 times for the initial model (“modINITIAL”) and one including only significant terms (“modFINAL”) for both biomass and density indices.

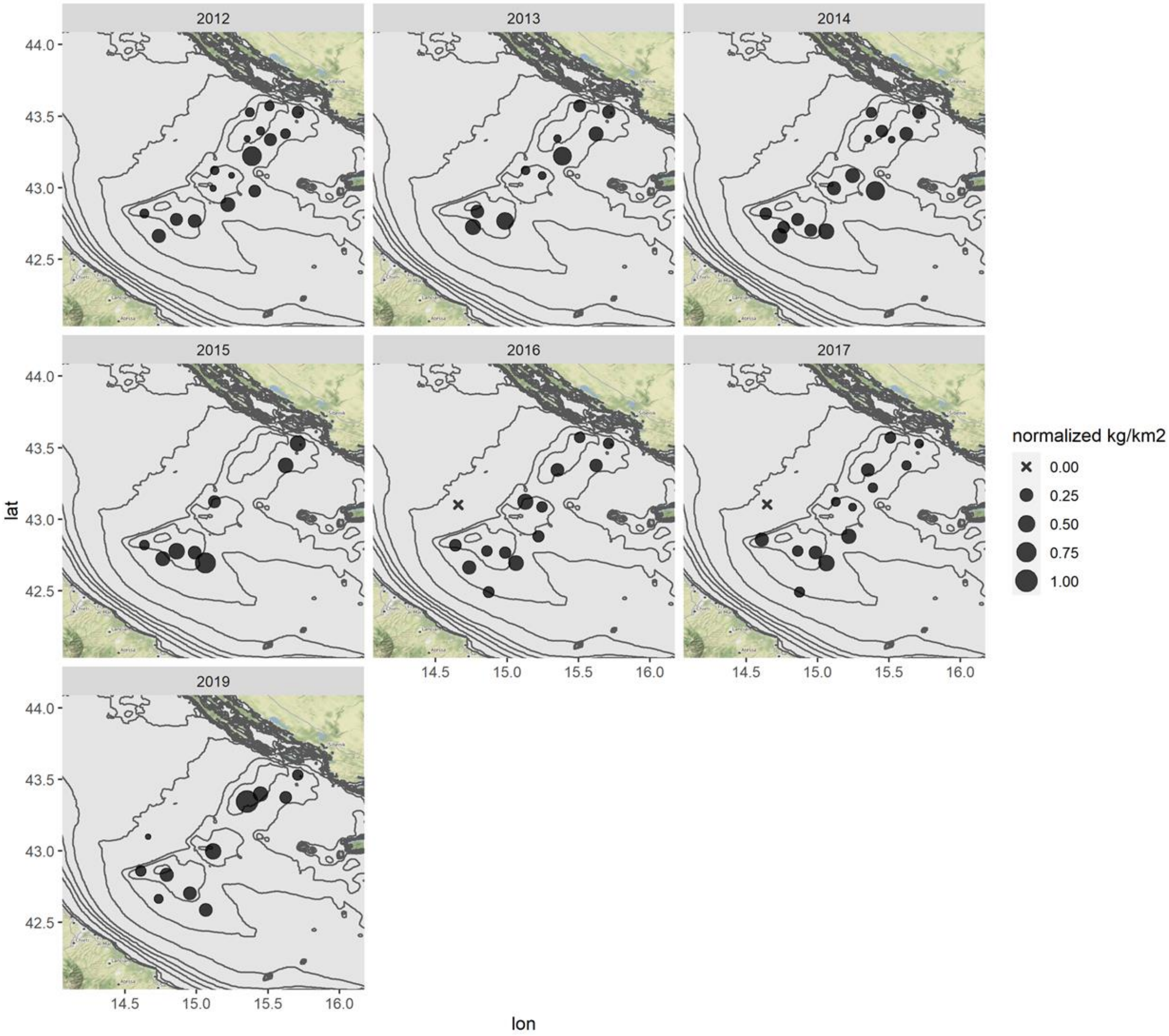
	Biomass index (kg/km^2)	Density index (N/km^2)
modINITIAL	21.155 ± 7.29	1834.191 ± 935.640
modFINAL	20.727 ± 7.357	1755.832 ± 839.267

S7 Table. Percentages of deviance explained by each term of “mod FINAL” for both biomass (kg/km^2) and density (N/km^2) indices. Percentages (%) were calculated on the total explained deviance of each model according to Wood et al. [96]

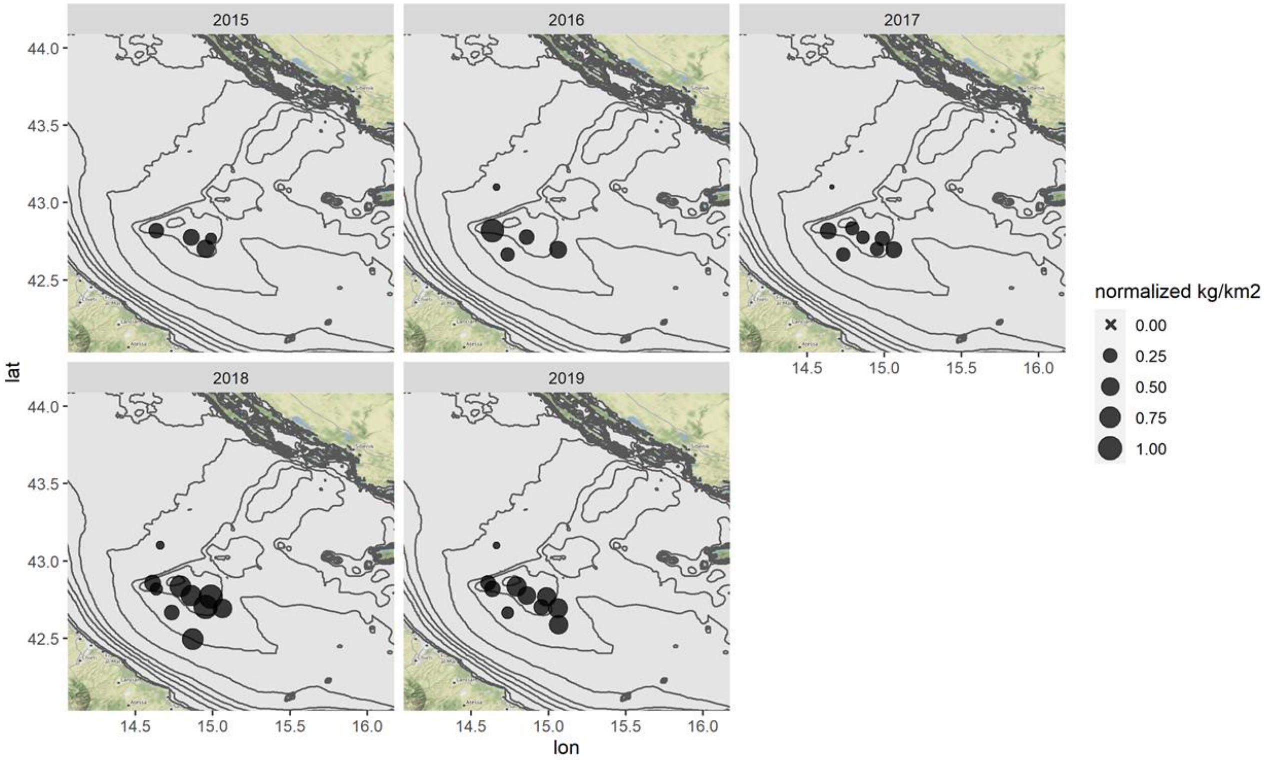
Covariate	Biomass index (kg/km^2)	Density index (N/km^2)
Yr:Fishery	33.4351145	34.83779972
Y	5.801526718	3.244005642
D	24.73282443	25.10578279
Oxy	6.870229008	8.74471086
Sal	7.938931298	10.1551481
ToD	10.38167939	7.757404795
Fishery	10.83969466	10.1551481



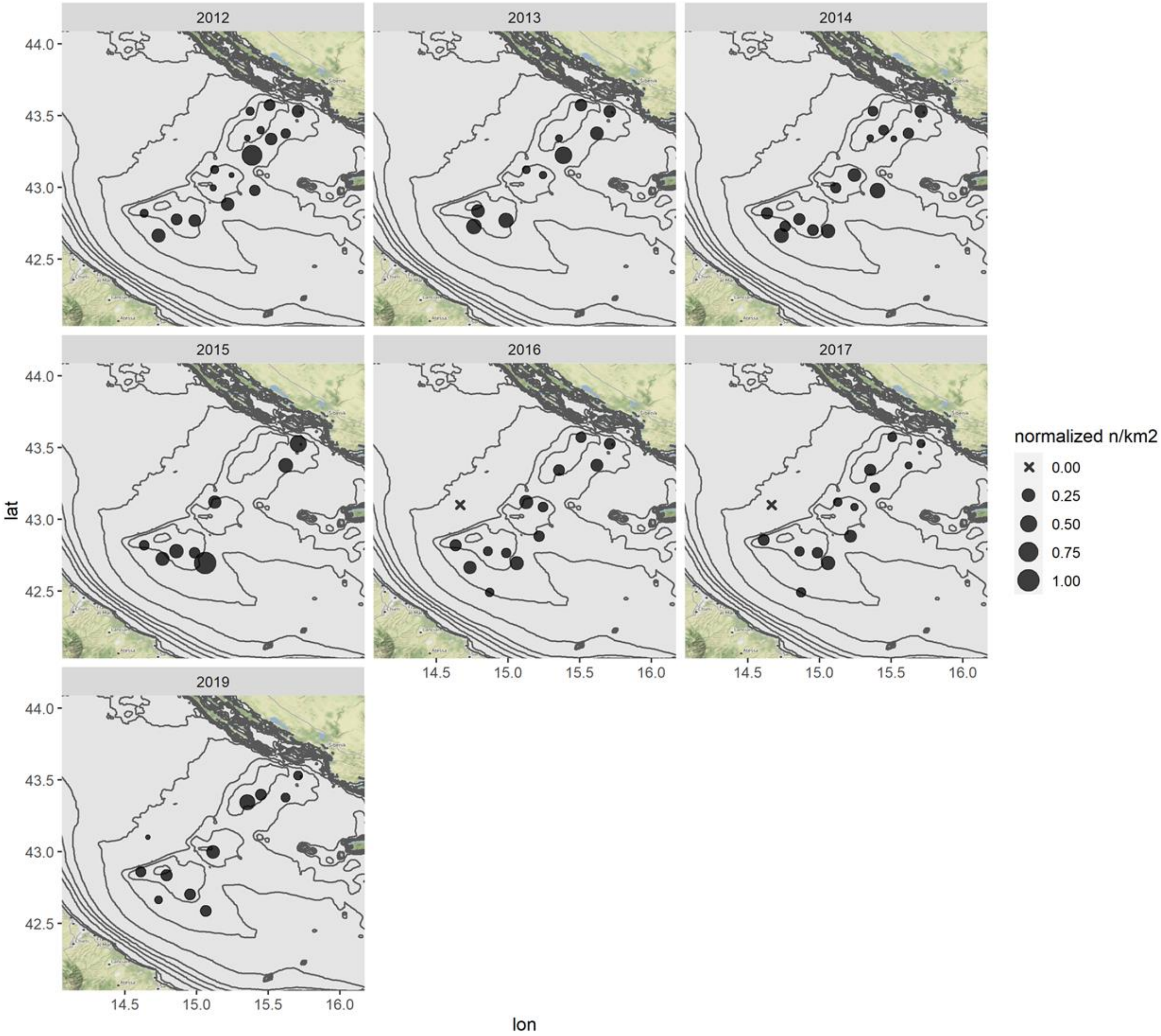
S1 Fig. Management measures implemented within the study area since July 2015. The maps show the bathymetry (source: [75]) and the boundaries of the study area, with western (orange polygon) and eastern (green polygon) sides divided by the Adriatic midline (source: [76]), and the spatial coverage of the management measures (inner red polygons) implemented over the years in the Pomo/Jabuka Pits. Panel a shows (red dense grid) the extension of the first partial closure to trawling activities from 26/07/2015 to 16/10/2016 (Italian Ministerial Decree n.162 03/07/2015; Croatian Government Ordinance n.1533 20/07/2015). Panel b presents the area (red sparse grid) subjected to limitation to fishery activities and the area (red dense grid) closed to trawling from 01/10/2016 to 31/08/2017 (Italian Ministerial Decree n.17064 19/10/2016; Croatian Government Ordinance n.1106 17/05/2017). Panel c reports the area closed to trawling activities (red dense grid) and the areas subjected to limitations to fishery (red sparse grids) from 01/09/2107 to 31/10/2017 (Italian Ministerial Decree n.466 01/06/2017). Panel d refers to the Fishery Restricted Area implemented in the Pomo/Jabuka Pits by the GFCM/41/2017/3 Recommendation of October 2017, which establishes the fishery ban zone (A) and the two buffer zones subjected to limitations (B and C).



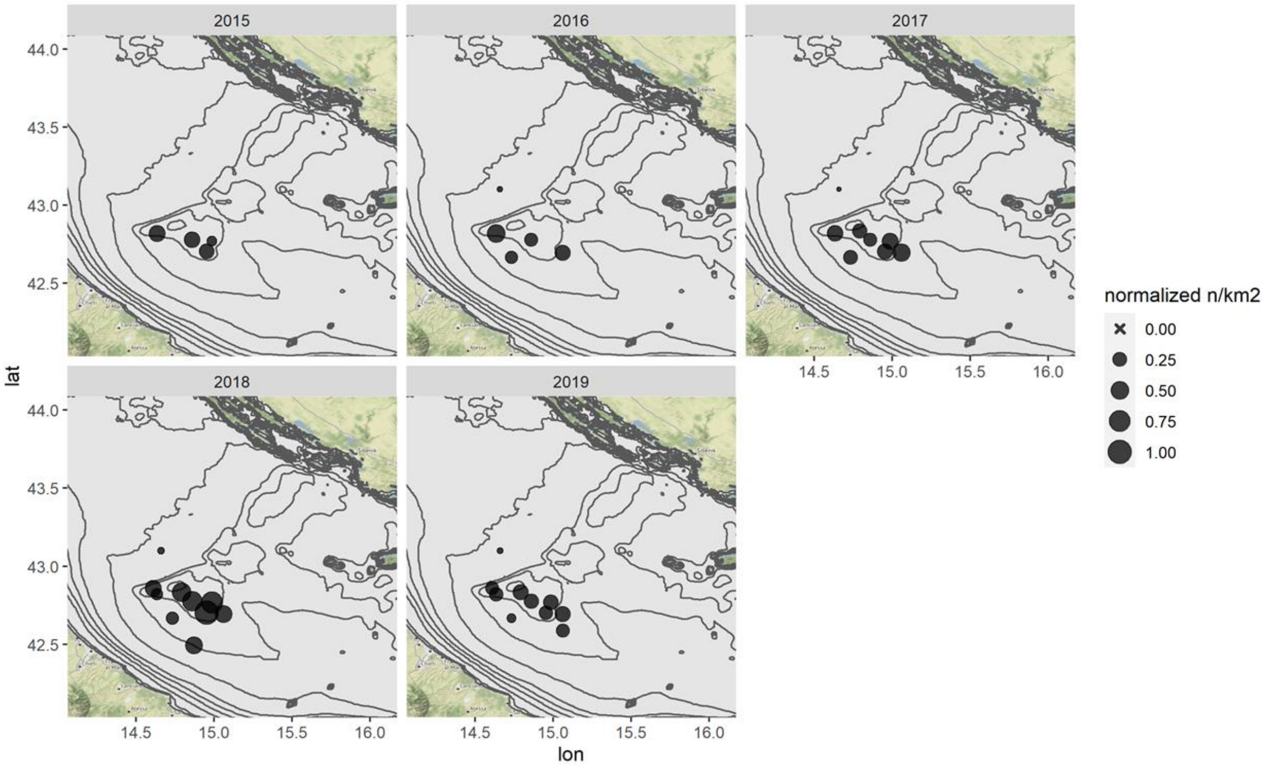
S2 Fig. Bubble Plots by year of normalized biomass CPUE calculated for each hauls of spring survey. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.



S3 Fig. Bubble Plots by year of normalized biomass CPUE calculated for each hauls of autumn survey. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.



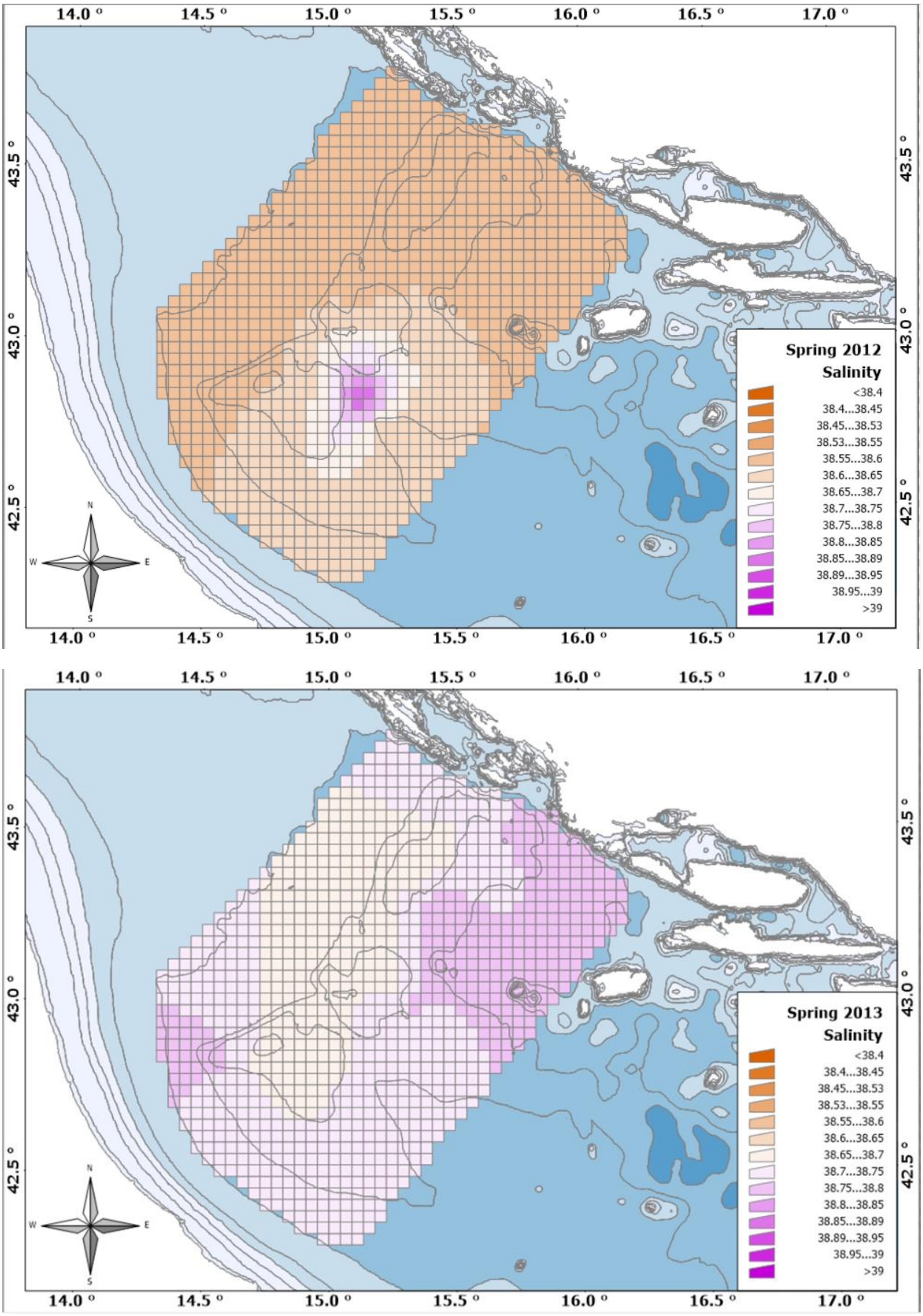
S4 Fig. Bubble Plots by year of normalized density CPUE calculated for each hauls of spring survey. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.

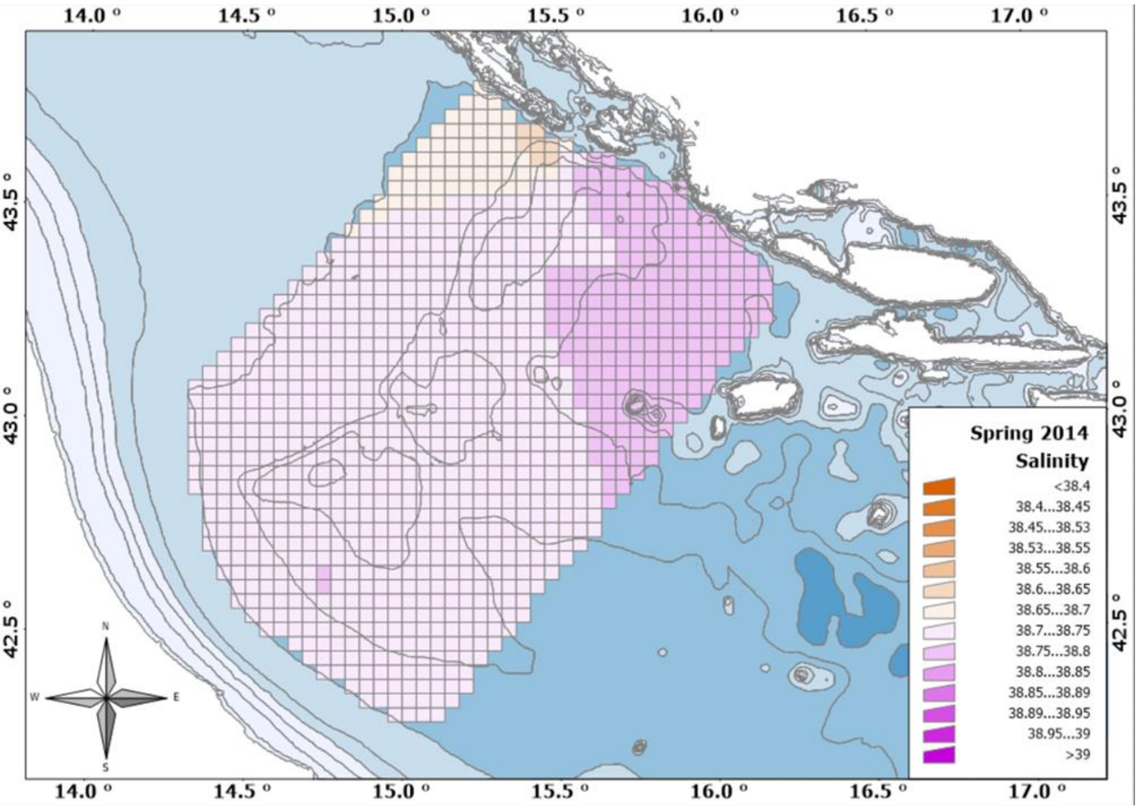


S5 Fig. Bubble Plots by year of normalized density CPUE calculated for each hauls of autumn survey. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.

Environmental maps

S6 Fig. Maps of bottom salinity values for the spring time series. Bathymetry layer source: [75].

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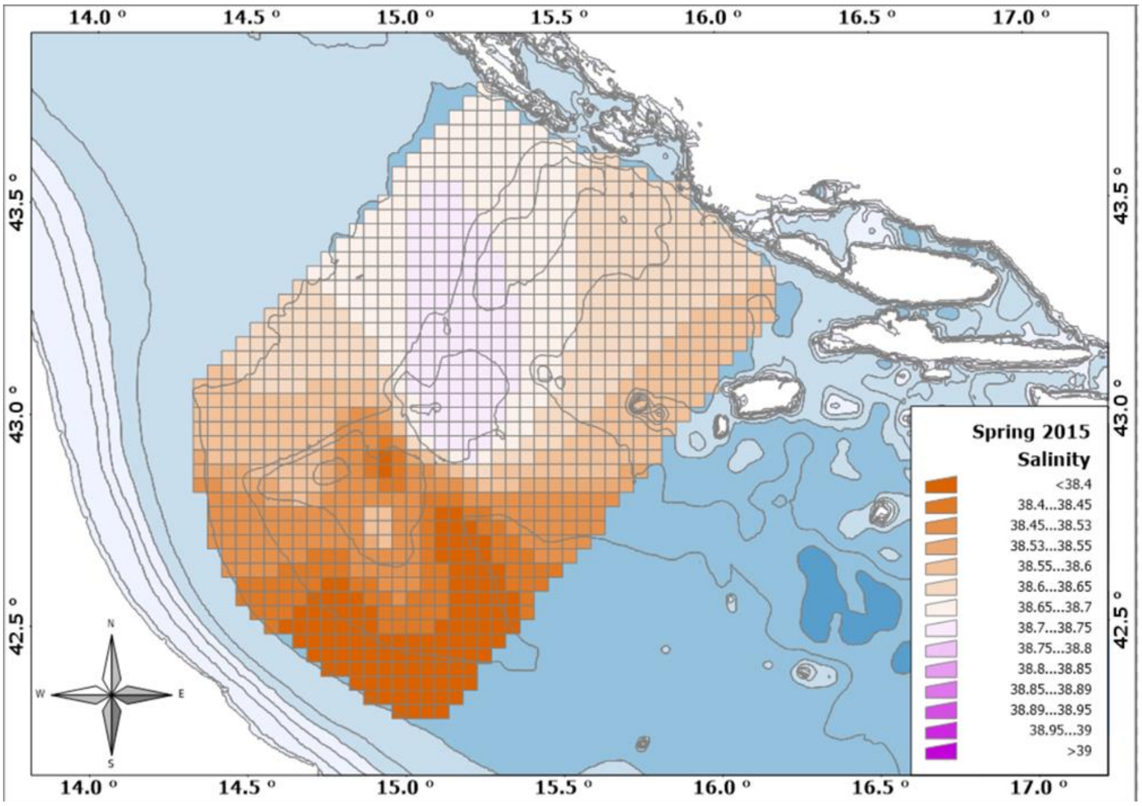
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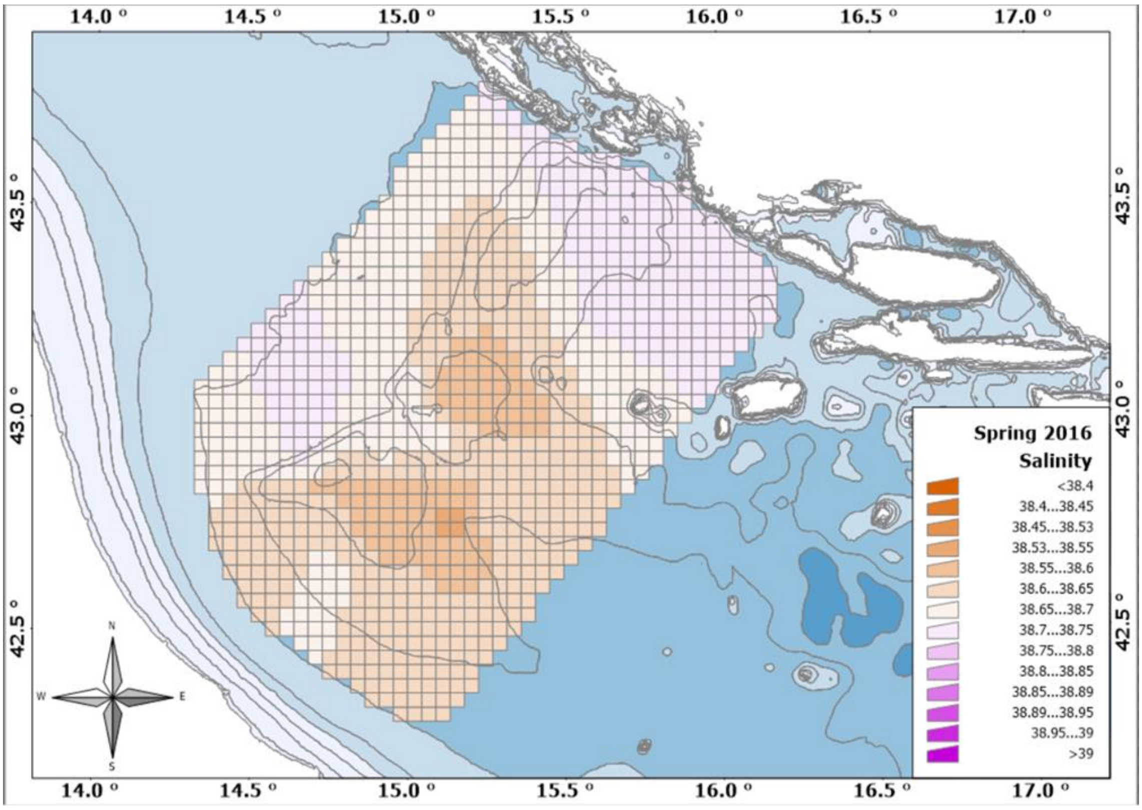
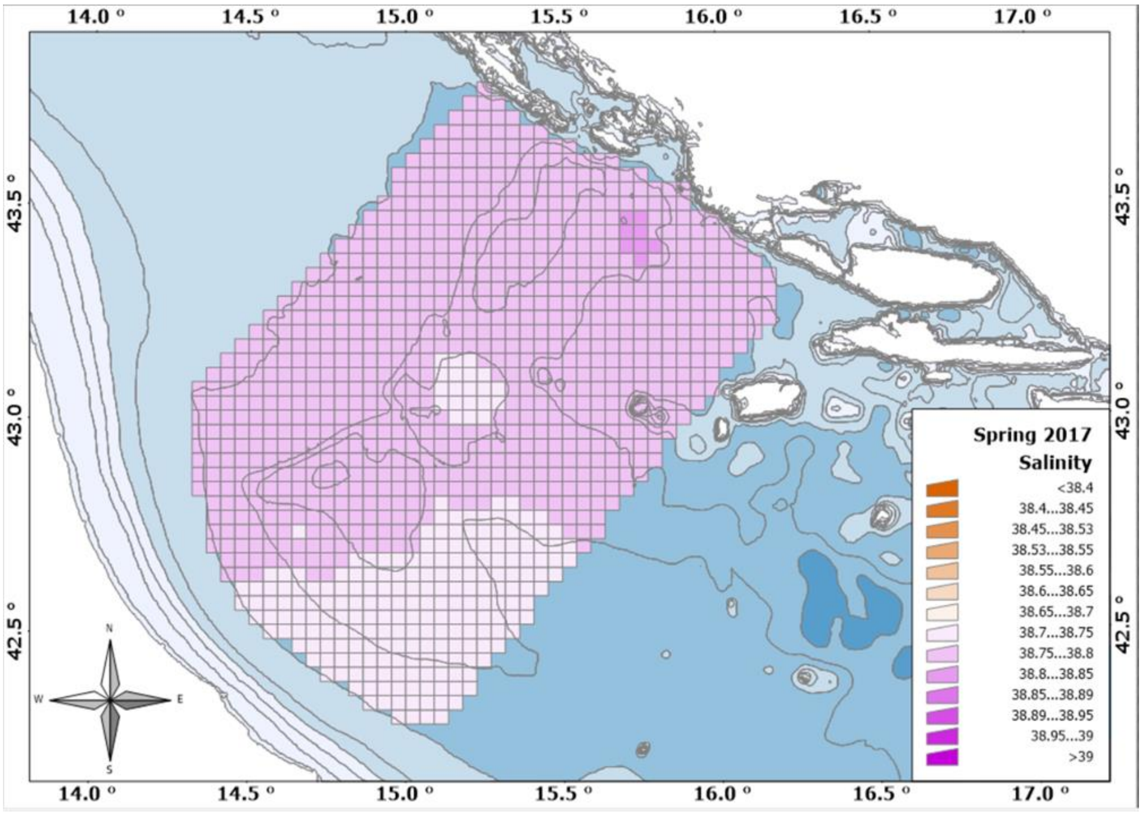
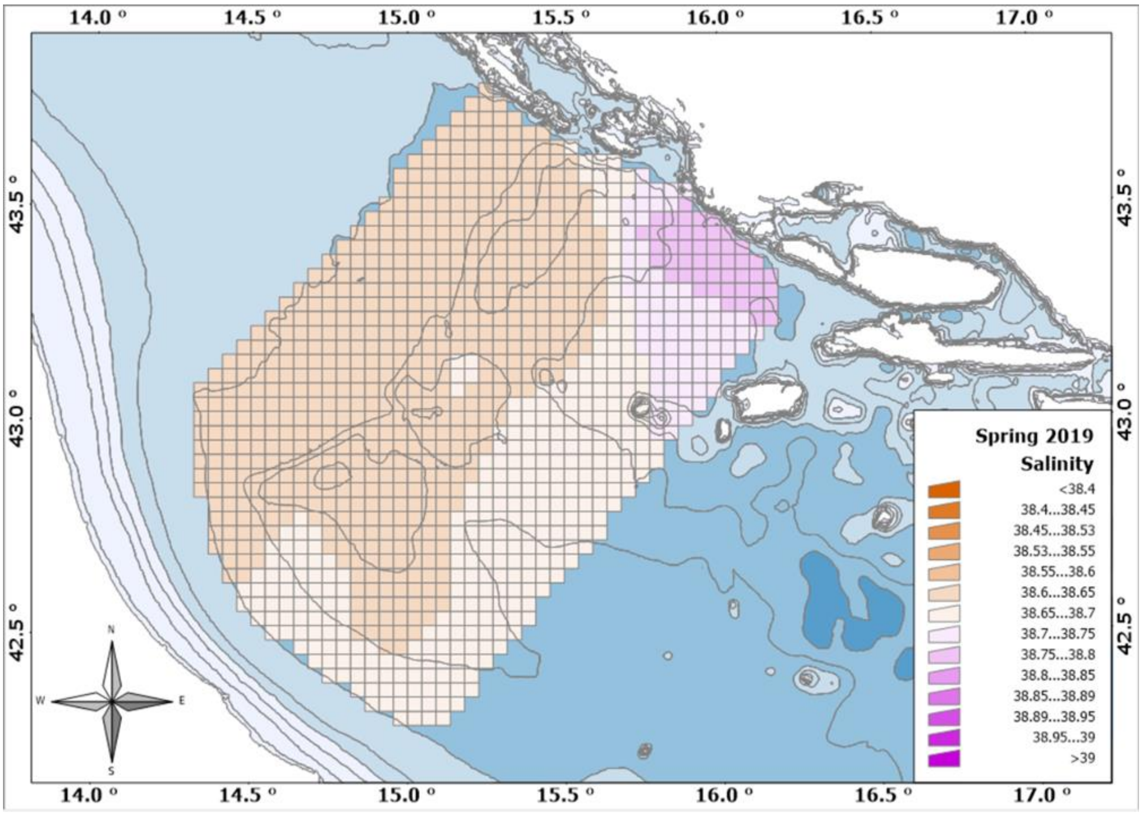
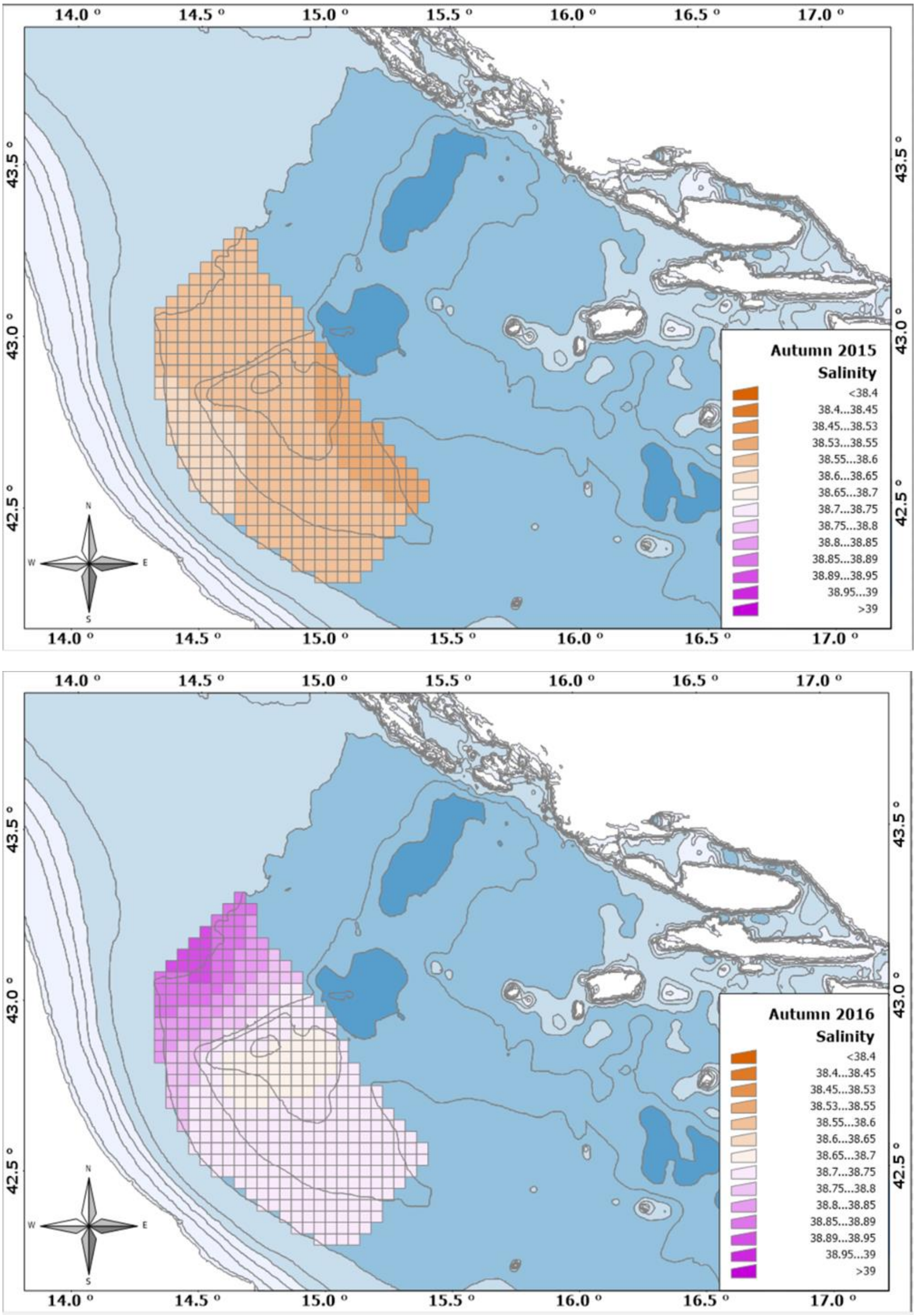
38.85...38.89
38.89...38.95
38.95...39
>39

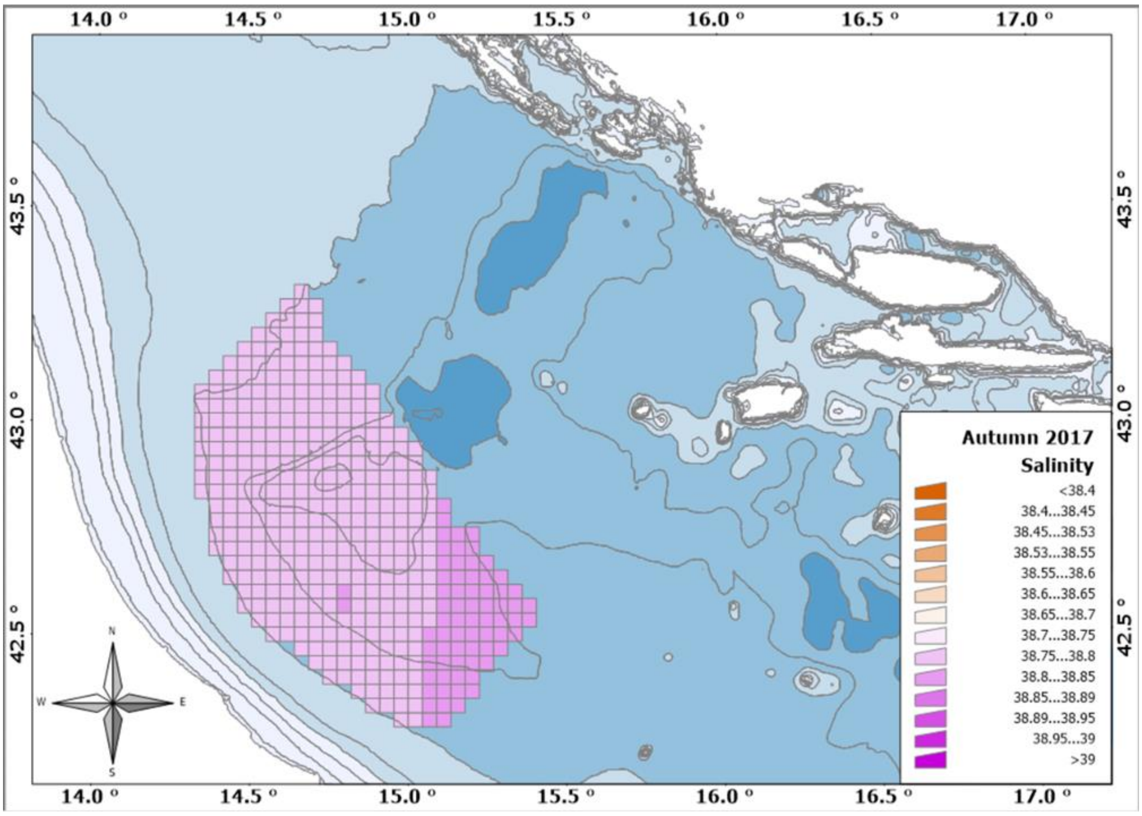

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
Spring 2016
Salinity
<38.4
38.4...38.45
38.45...38.53
38.53...38.55
38.55...38.6
38.6...38.65
38.65...38.7
38.7...38.75
38.75...38.8
38.8...38.85
38.85...38.89
38.89...38.95
38.95...39
>39
N
W
E
S

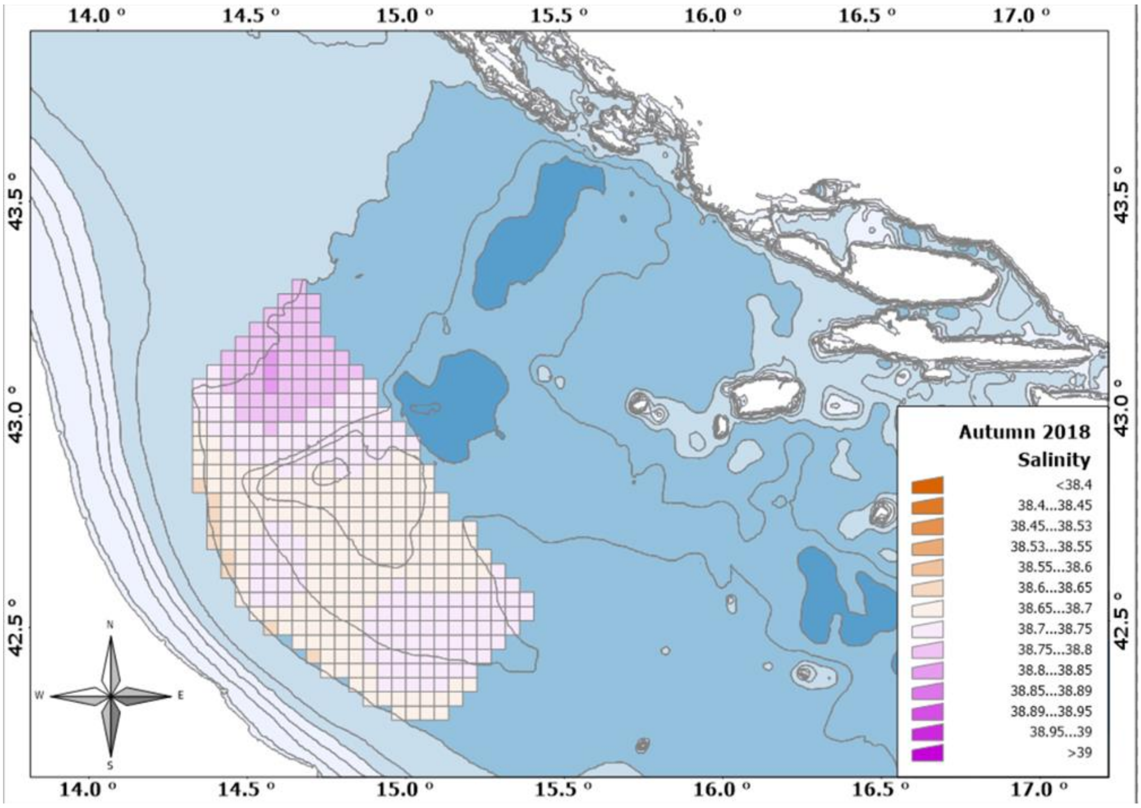

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
Spring 2017
Salinity
<38.4
38.4...38.45
38.45...38.53
38.53...38.55
38.55...38.6
38.6...38.65
38.65...38.7
38.7...38.75
38.75...38.8
38.8...38.85
38.85...38.89
38.89...38.95
38.95...39
>39
N
W
E
S

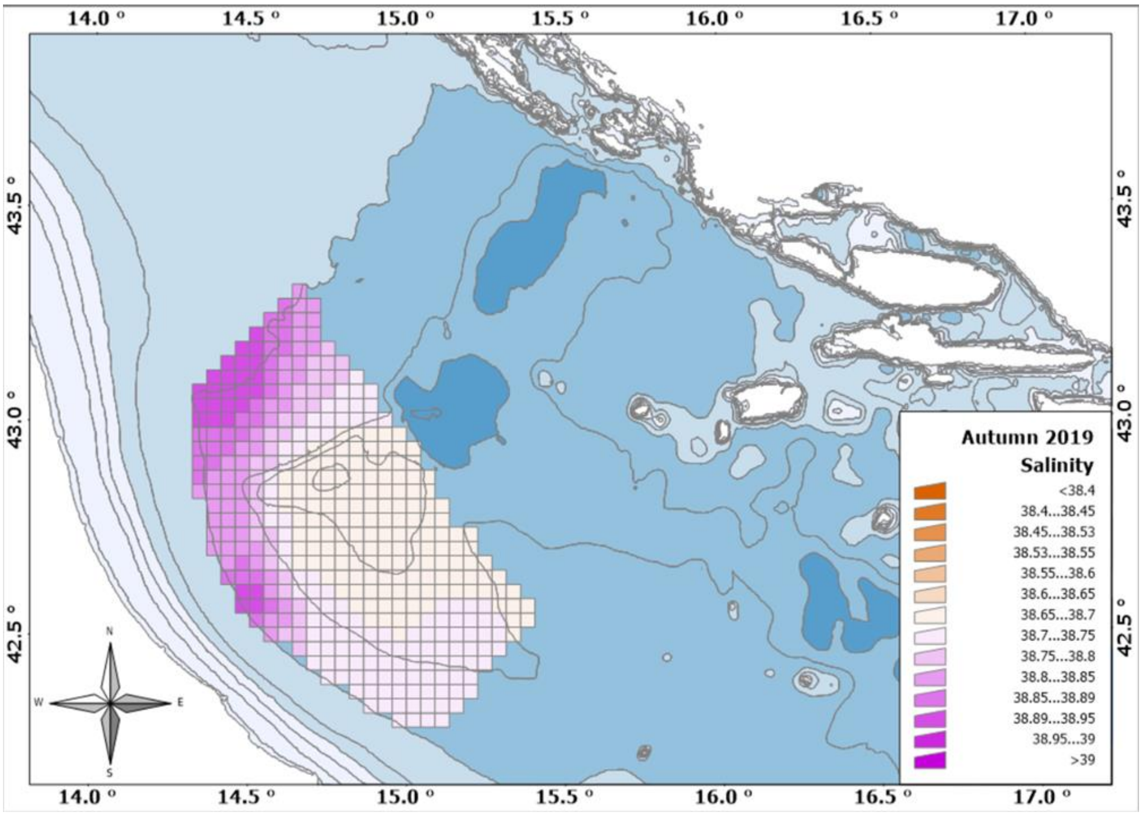

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
S
W
E
Spring 2019
Salinity
<38.4
38.4...38.45
38.45...38.53
38.53...38.55
38.55...38.6
38.6...38.65
38.65...38.7
38.7...38.75
38.75...38.8
38.8...38.85
38.85...38.89
38.89...38.95
38.95...39
>39

S7 Fig. Maps of bottom salinity values for the autumn time series. Bathymetry layer source: [75].



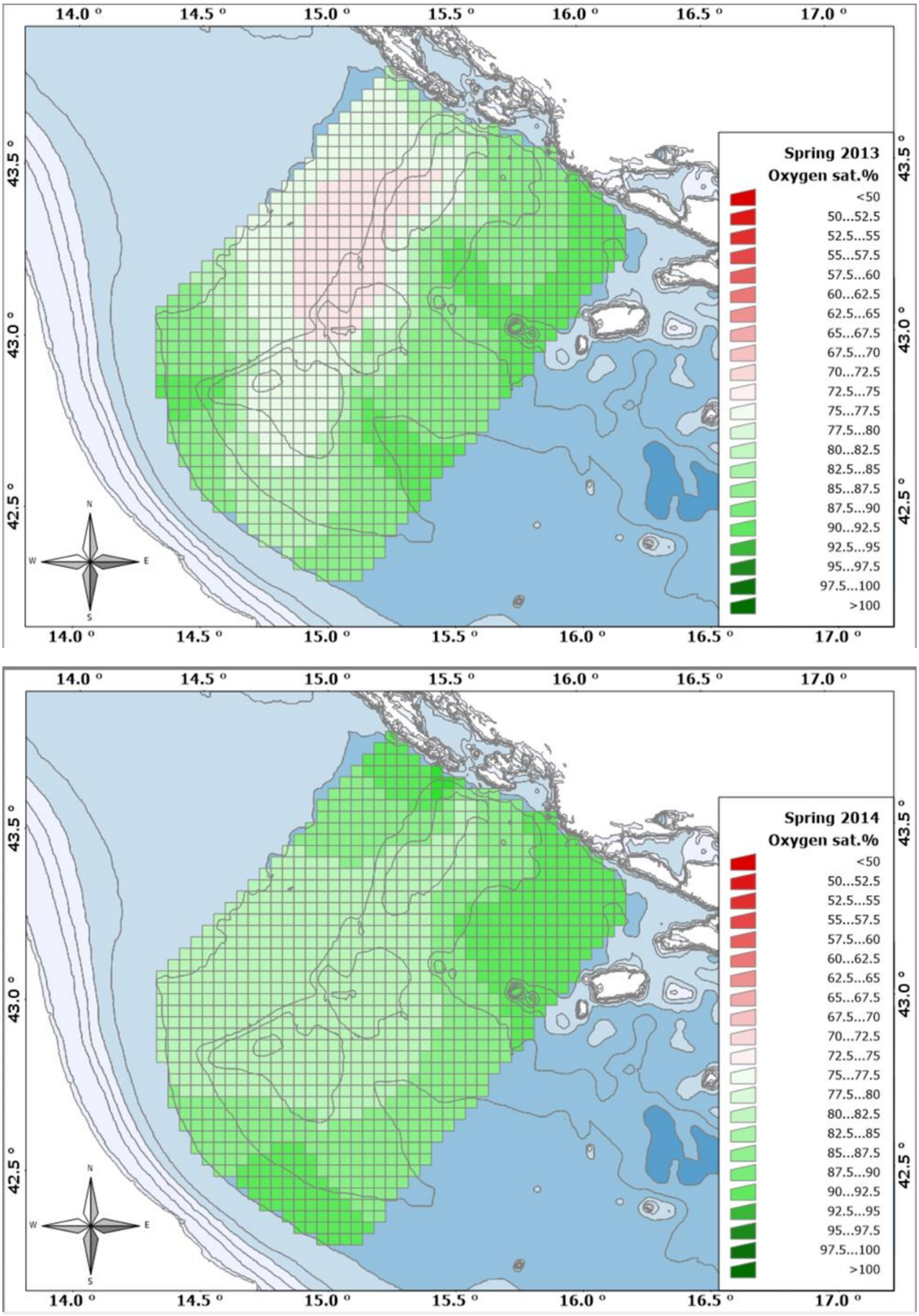

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Autumn 2017
Salinity
<38.4
38.4...38.45
38.45...38.53
38.53...38.55
38.55...38.6
38.6...38.65
38.65...38.7
38.7...38.75
38.75...38.8
38.8...38.85
38.85...38.89
38.89...38.95
38.95...39
>39

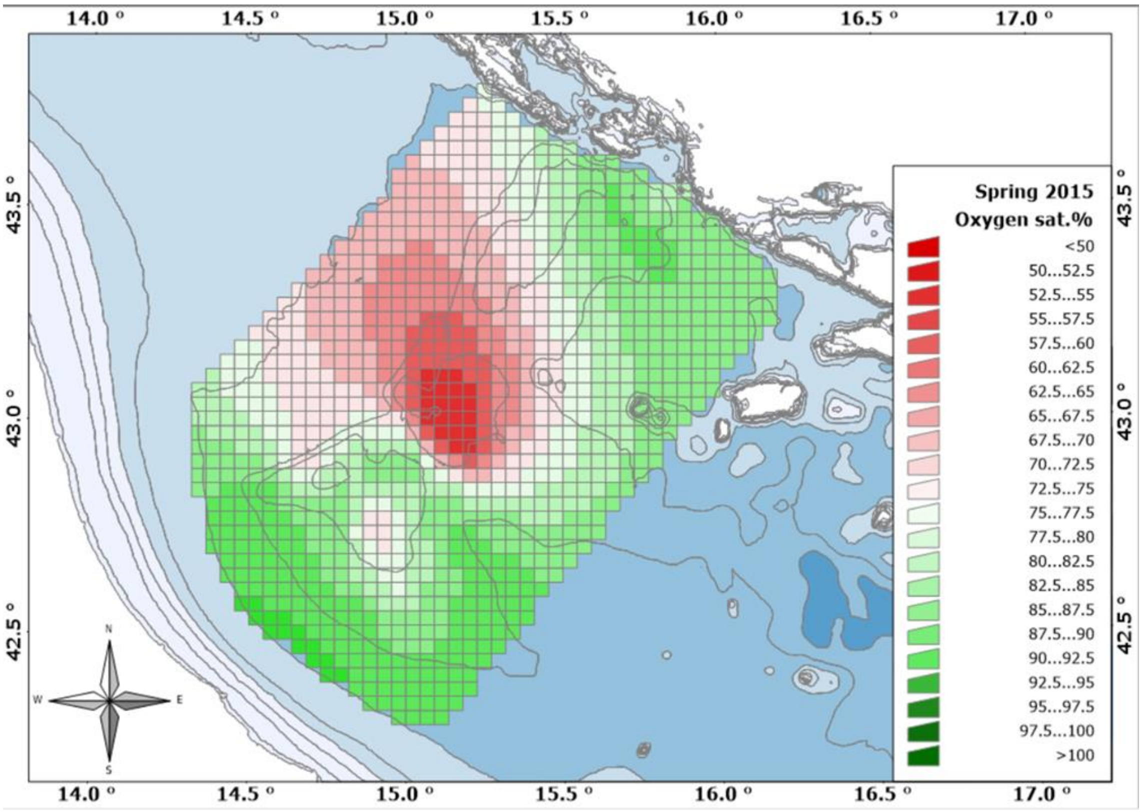

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Autumn 2018
Salinity
<38.4
38.4...38.45
38.45...38.53
38.53...38.55
38.55...38.6
38.6...38.65
38.65...38.7
38.7...38.75
38.75...38.8
38.8...38.85
38.85...38.89
38.89...38.95
38.95...39
>39

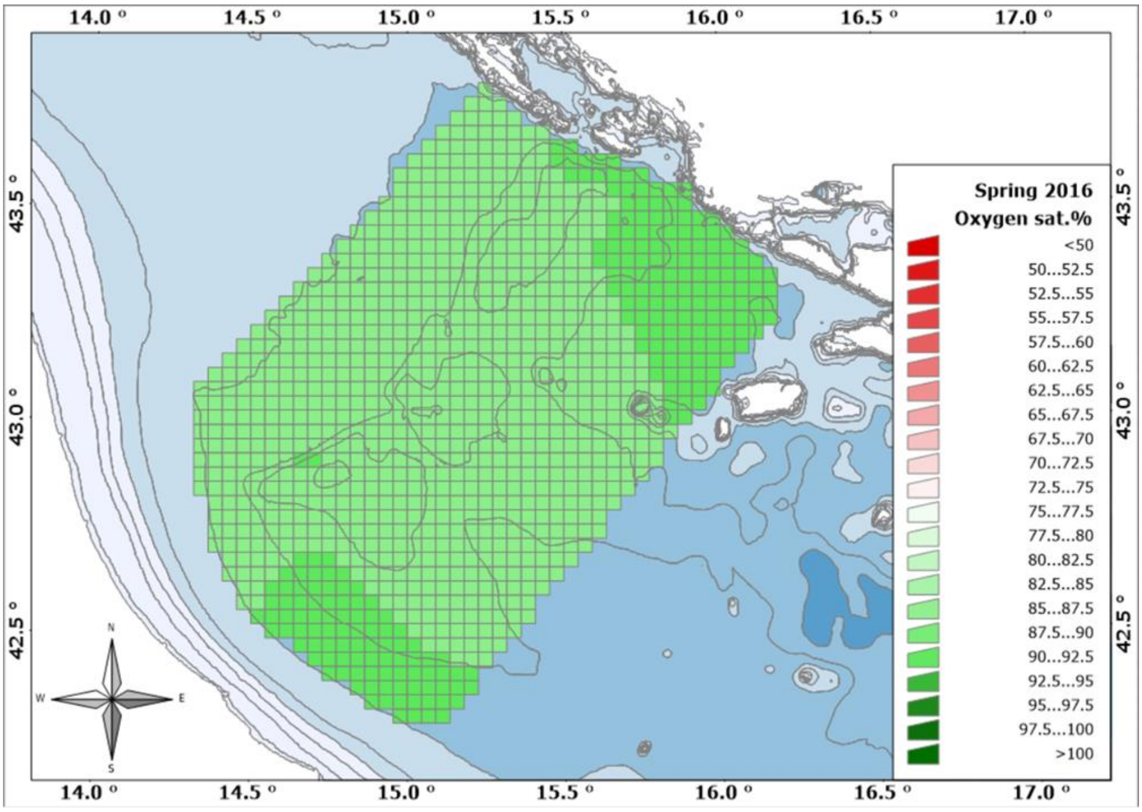

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Autumn 2019
Salinity
<38.4
38.4...38.45
38.45...38.53
38.53...38.55
38.55...38.6
38.6...38.65
38.65...38.7
38.7...38.75
38.75...38.8
38.8...38.85
38.85...38.89
38.89...38.95
38.95...39
>39

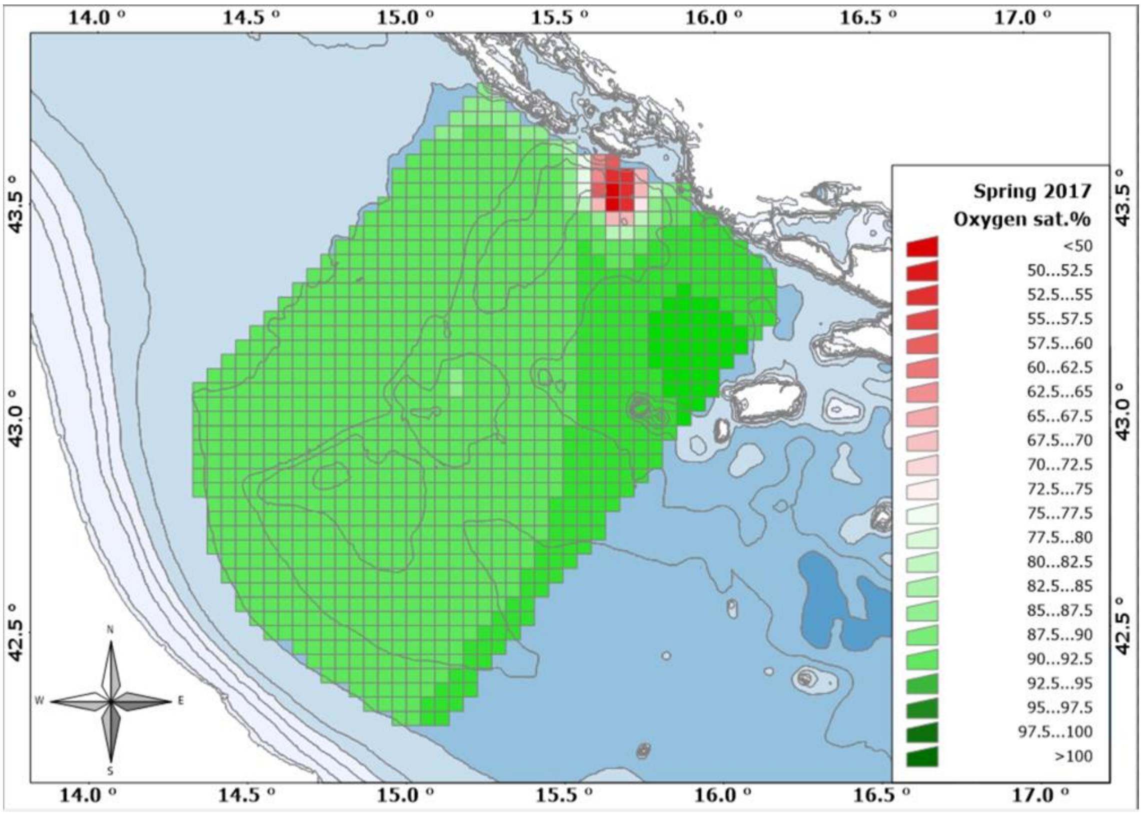
S8 Fig. Maps of bottom dissolved oxygen saturation percentage for the spring time series.

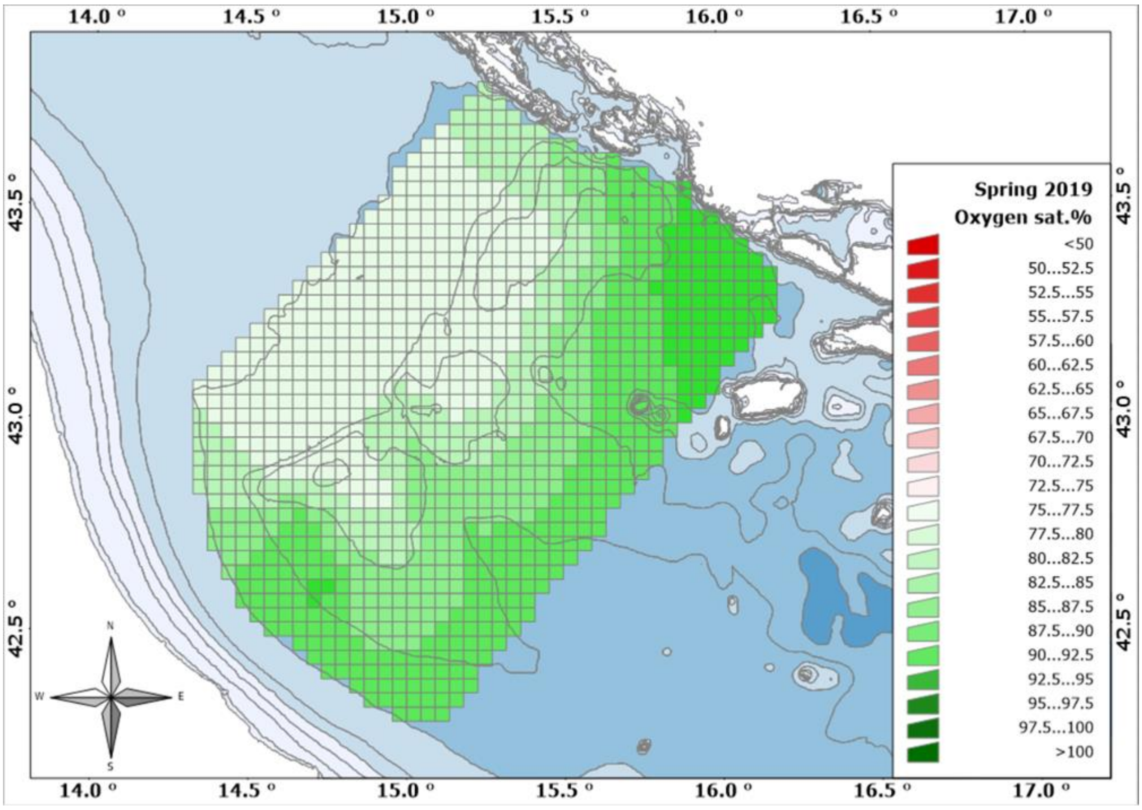
Bathymetry layer source: [75].




14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Spring 2015
Oxygen sat.%
<50
50...52.5
52.5...55
55...57.5
57.5...60
60...62.5
62.5...65
65...67.5
67.5...70
70...72.5
72.5...75
75...77.5
77.5...80
80...82.5
82.5...85
85...87.5
87.5...90
90...92.5
92.5...95
95...97.5
97.5...100
>100

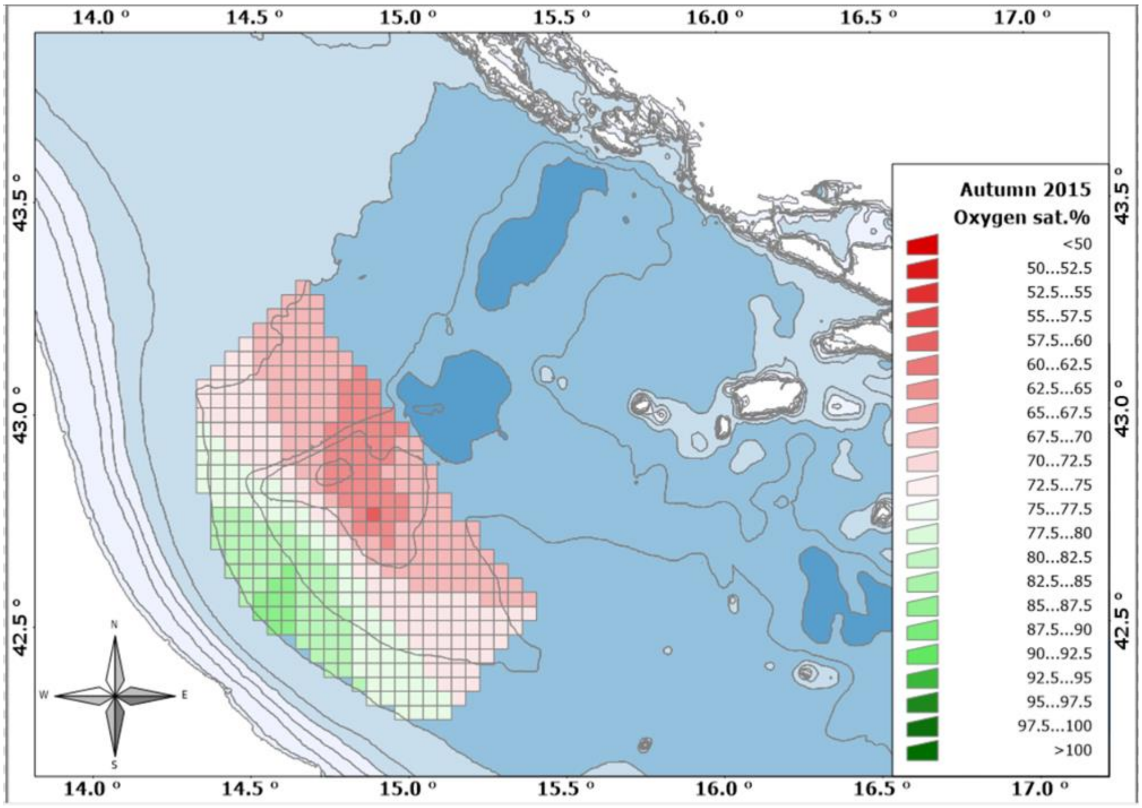

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Spring 2016
Oxygen sat.%
<50
50...52.5
52.5...55
55...57.5
57.5...60
60...62.5
62.5...65
65...67.5
67.5...70
70...72.5
72.5...75
75...77.5
77.5...80
80...82.5
82.5...85
85...87.5
87.5...90
90...92.5
92.5...95
95...97.5
97.5...100
>100

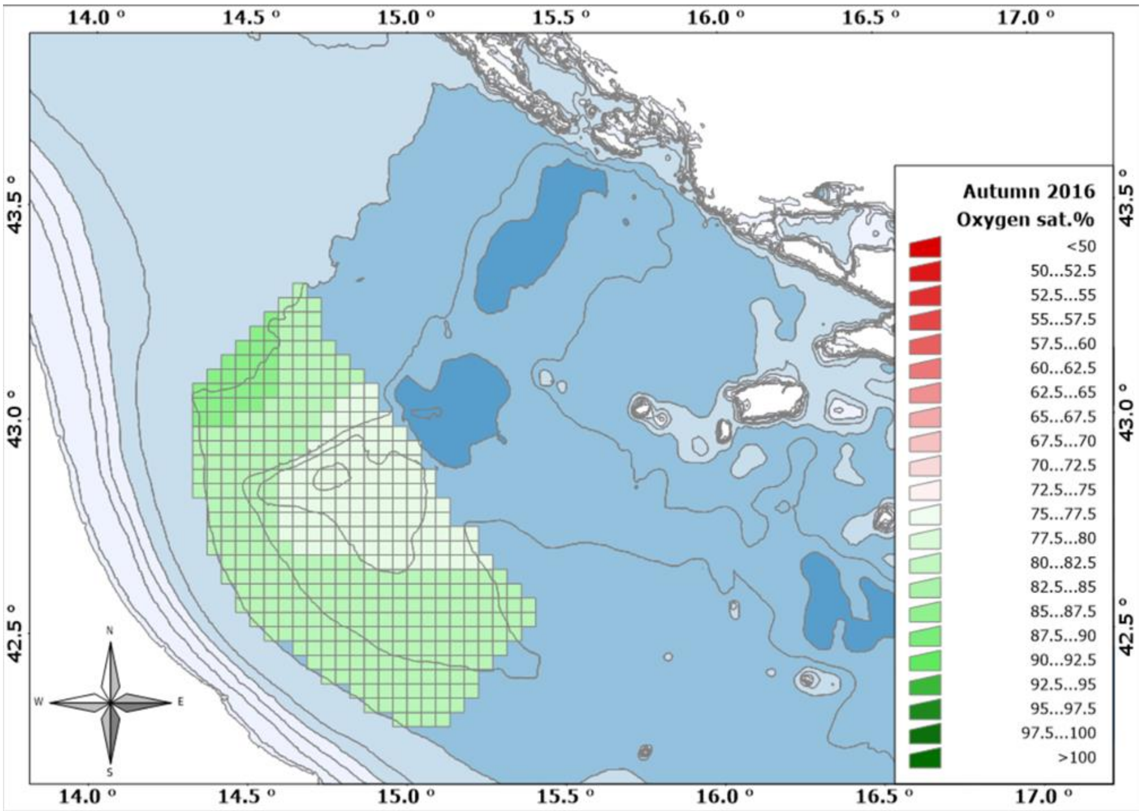

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Spring 2017
Oxygen sat.%
<50
50...52.5
52.5...55
55...57.5
57.5...60
60...62.5
62.5...65
65...67.5
67.5...70
70...72.5
72.5...75
75...77.5
77.5...80
80...82.5
82.5...85
85...87.5
87.5...90
90...92.5
92.5...95
95...97.5
97.5...100
>100

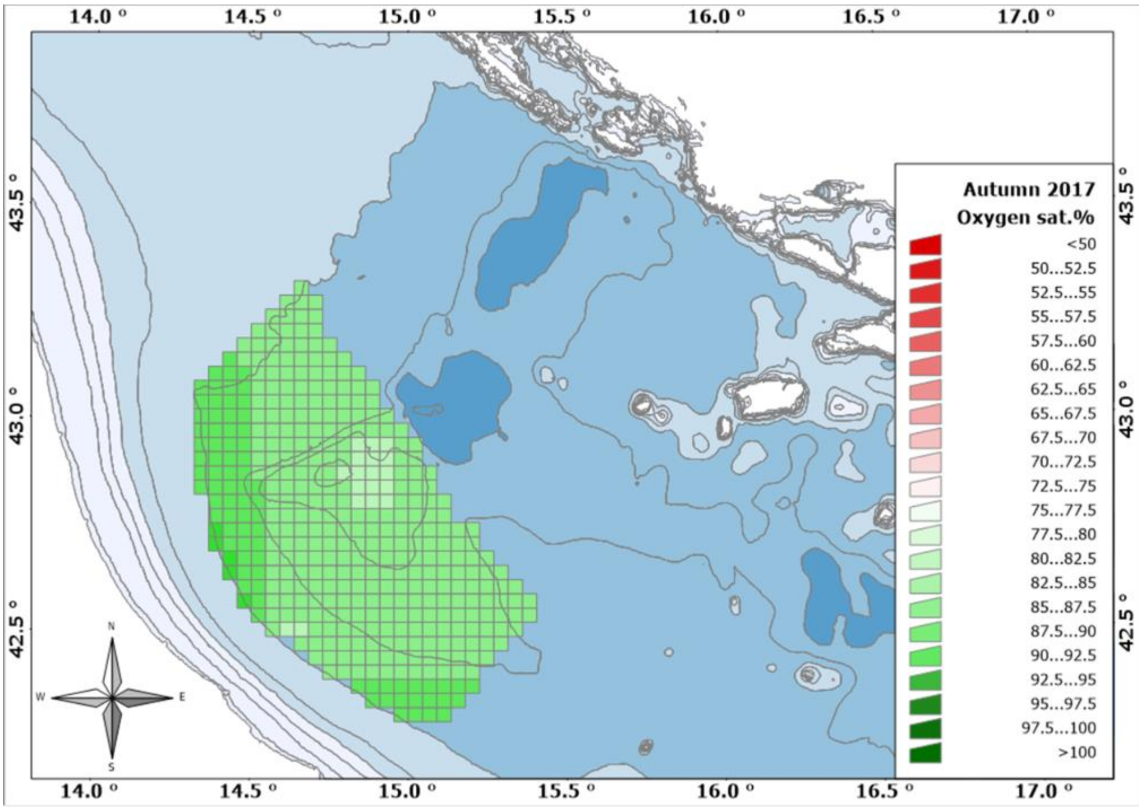

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Spring 2019
Oxygen sat.%
<50
50...52.5
52.5...55
55...57.5
57.5...60
60...62.5
62.5...65
65...67.5
67.5...70
70...72.5
72.5...75
75...77.5
77.5...80
80...82.5
82.5...85
85...87.5
87.5...90
90...92.5
92.5...95
95...97.5
97.5...100
>100

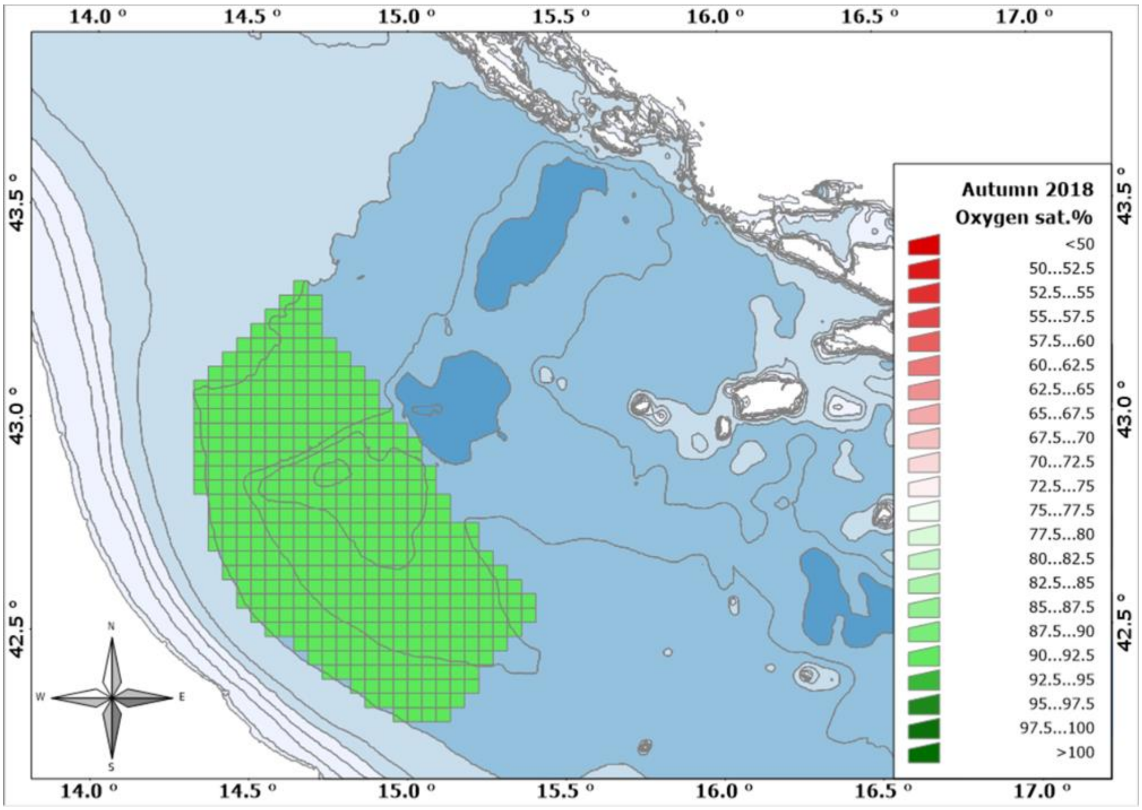
S9 Fig. Maps of bottom dissolved oxygen saturation percentage for the autumn time series.

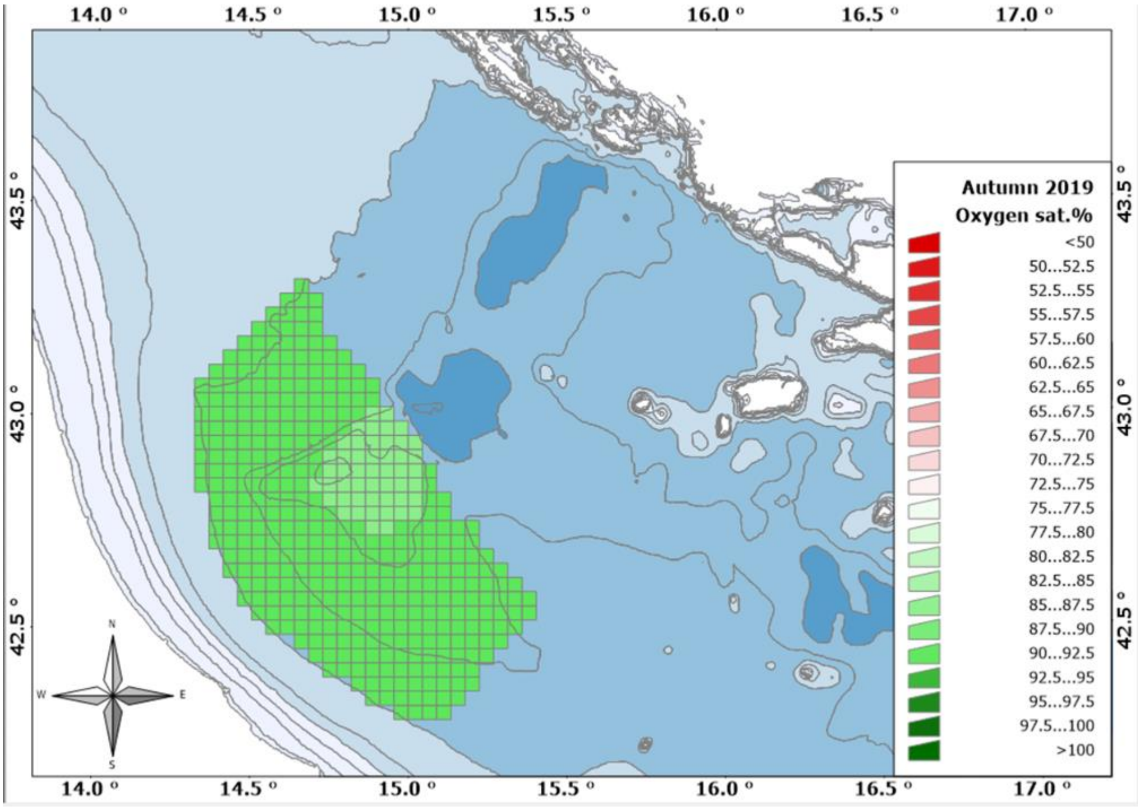
Bathymetry layer source: [75].



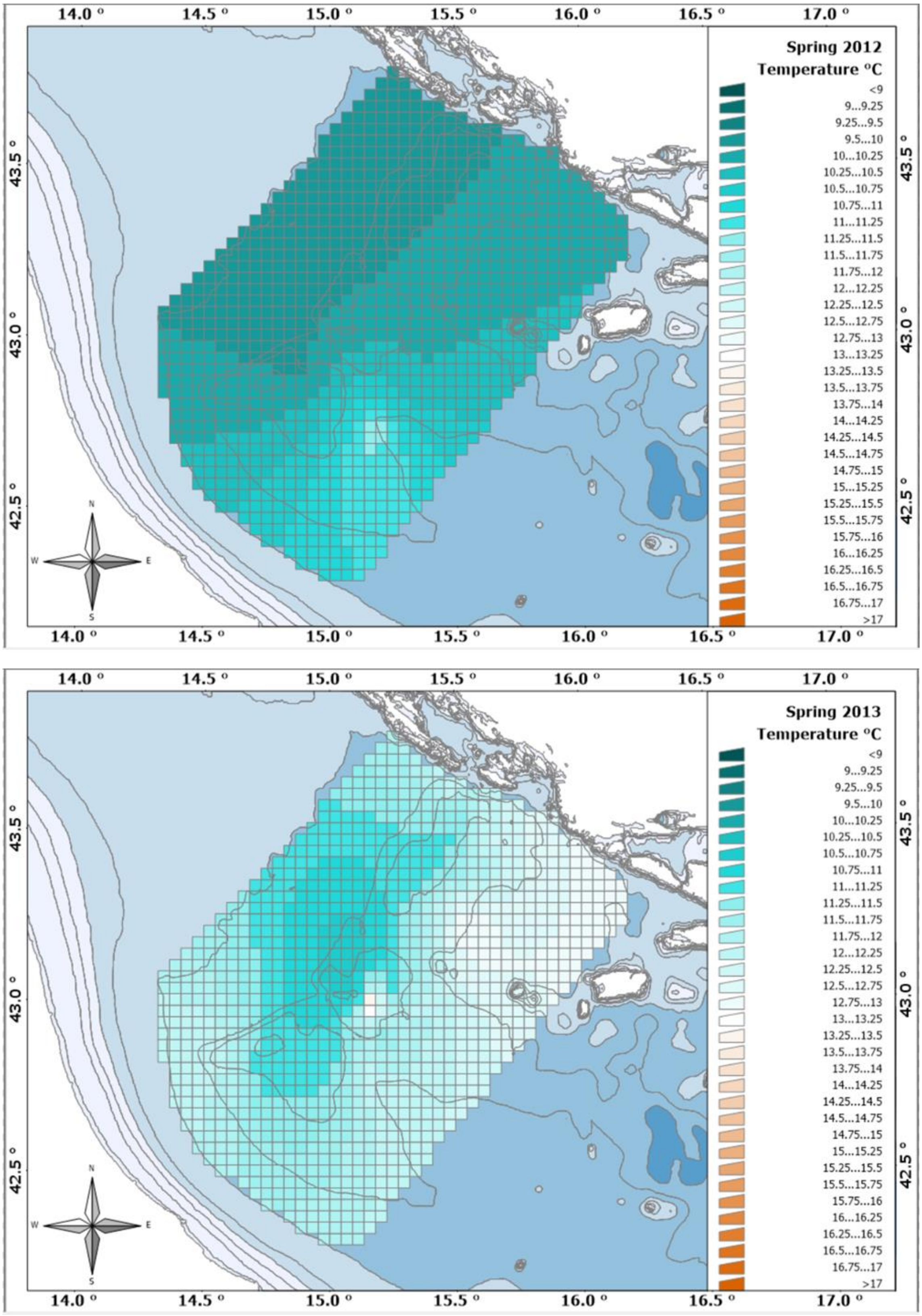


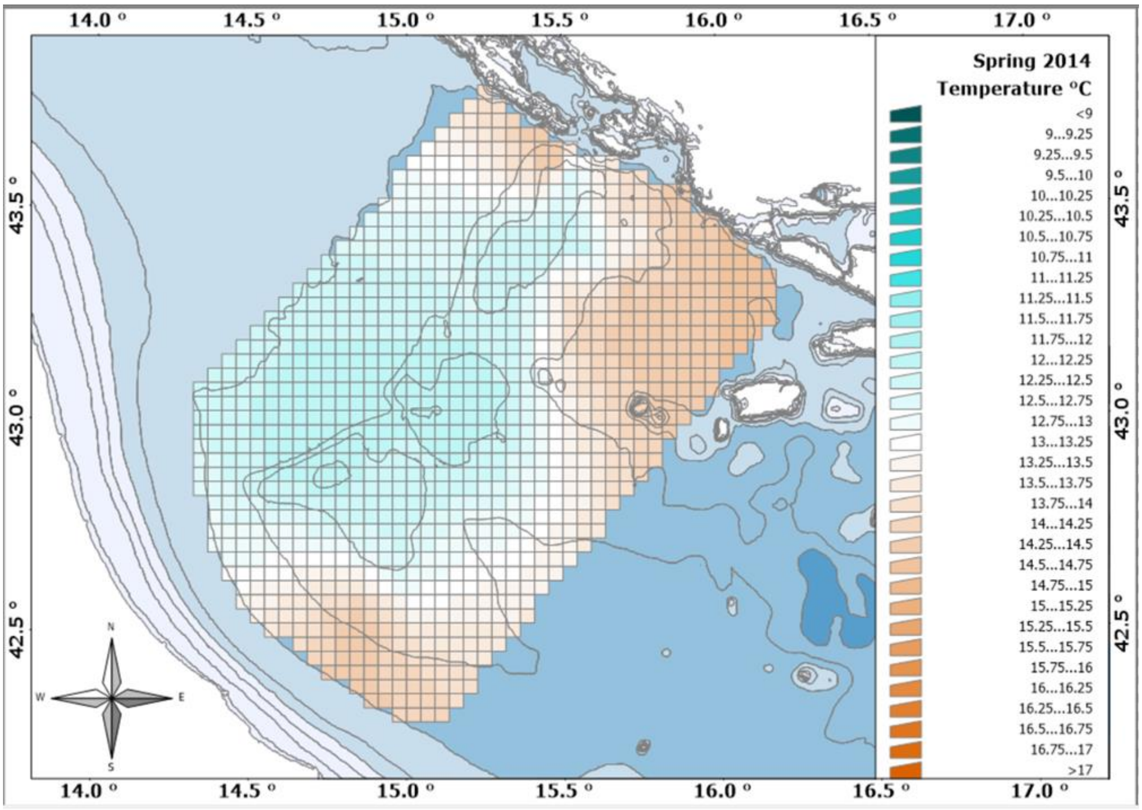

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Autumn 2017
Oxygen sat.%
<50
50...52.5
52.5...55
55...57.5
57.5...60
60...62.5
62.5...65
65...67.5
67.5...70
70...72.5
72.5...75
75...77.5
77.5...80
80...82.5
82.5...85
85...87.5
87.5...90
90...92.5
92.5...95
95...97.5
97.5...100
>100

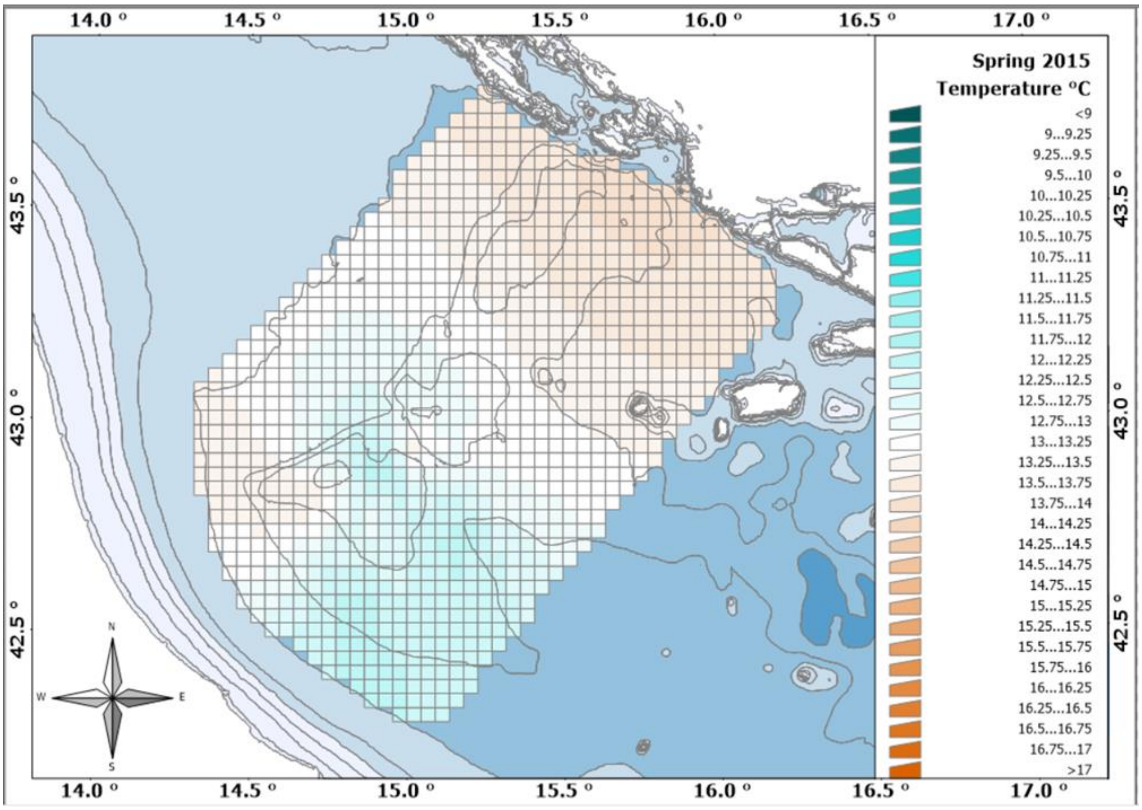

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S
Autumn 2018
Oxygen sat.%
<50
50...52.5
52.5...55
55...57.5
57.5...60
60...62.5
62.5...65
65...67.5
67.5...70
70...72.5
72.5...75
75...77.5
77.5...80
80...82.5
82.5...85
85...87.5
87.5...90
90...92.5
92.5...95
95...97.5
97.5...100
>100

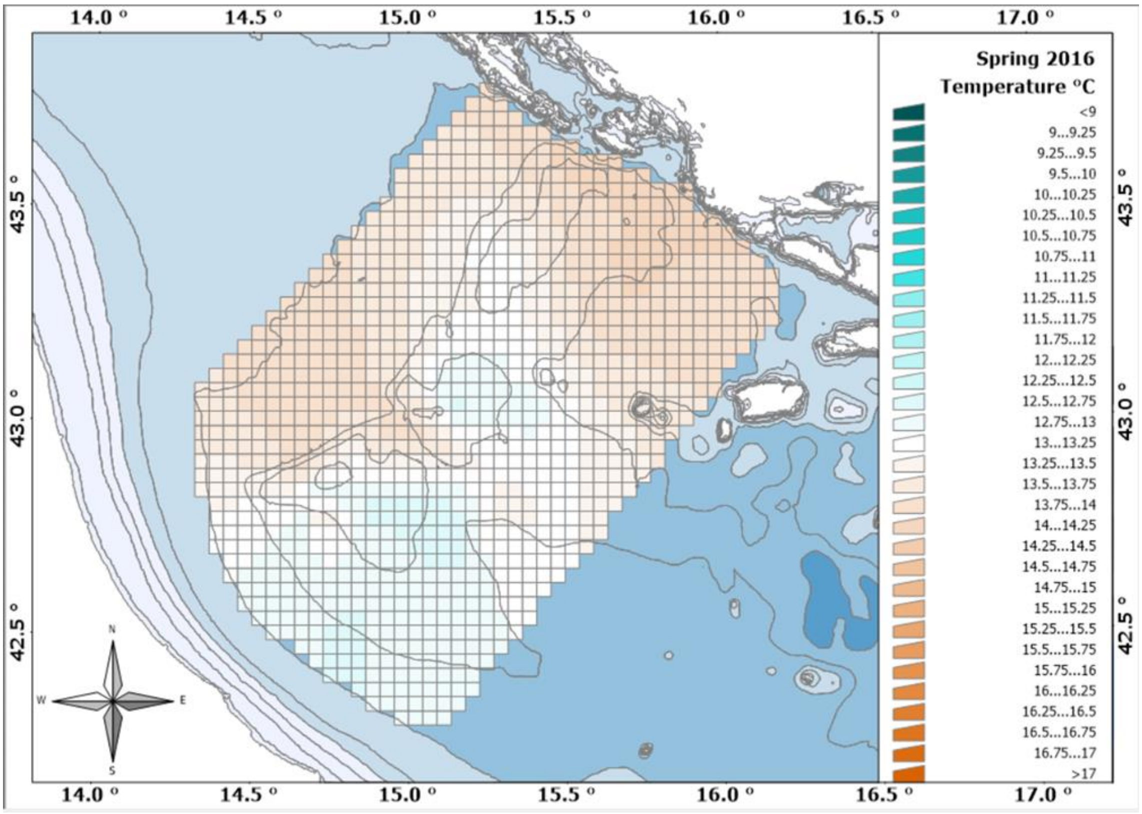

14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
Autumn 2019
Oxygen sat.%
<50
50...52.5
52.5...55
55...57.5
57.5...60
60...62.5
62.5...65
65...67.5
67.5...70
70...72.5
72.5...75
75...77.5
77.5...80
80...82.5
82.5...85
85...87.5
87.5...90
90...92.5
92.5...95
95...97.5
97.5...100
>100
N
E
S
W

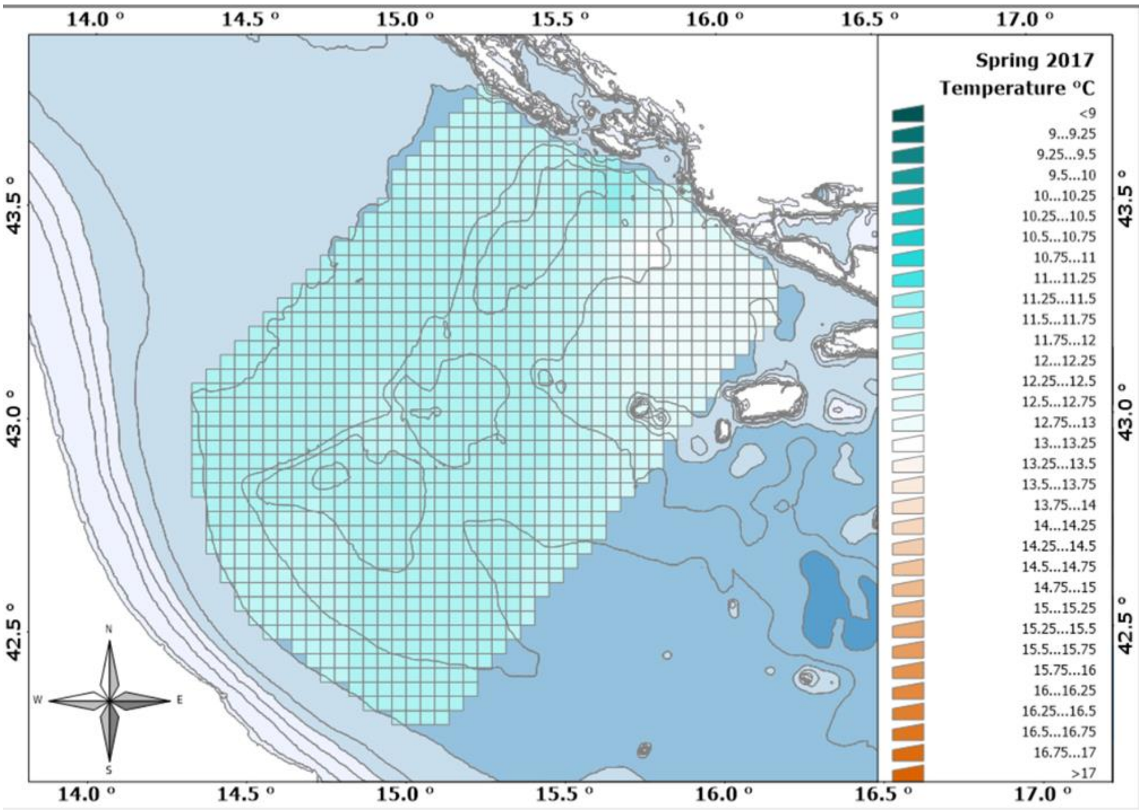
S10 Fig. Maps of bottom temperature values for the spring time series. Bathymetry layer source: [75].

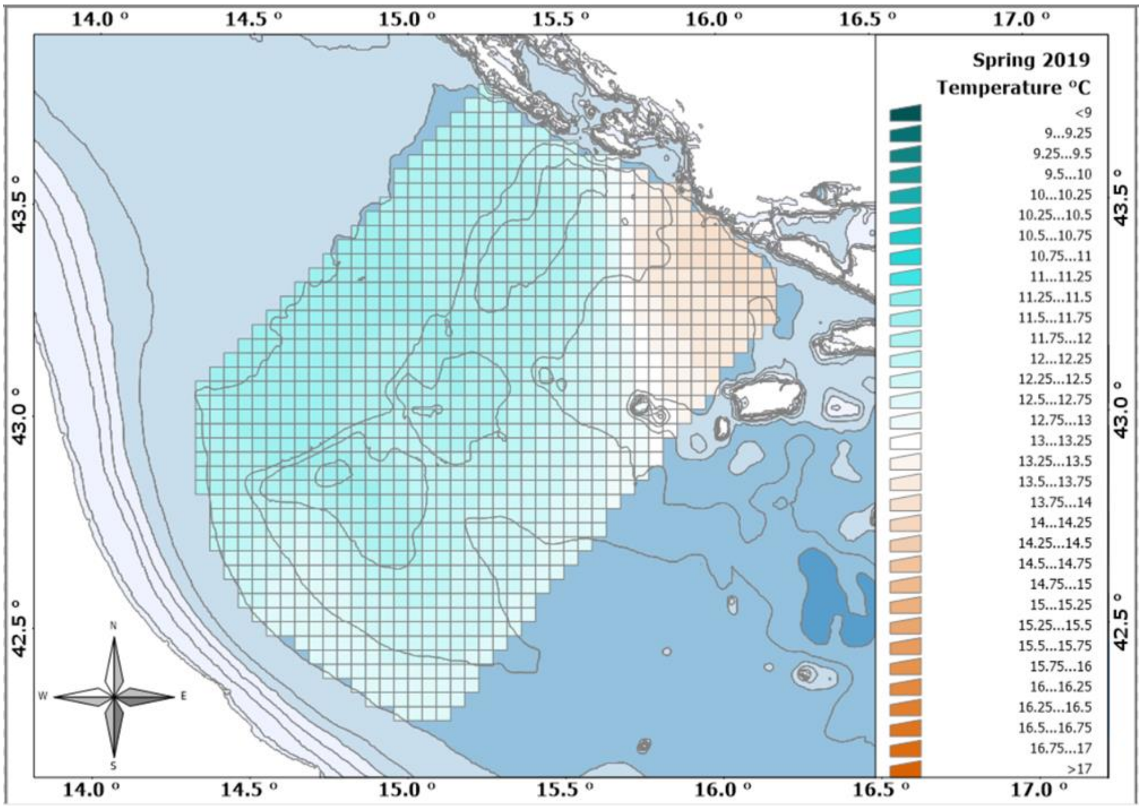



Spring 2014
Temperature °C
<9
9...9.25
9.25...9.5
9.5...10
10...10.25
10.25...10.5
10.5...10.75
10.75...11
11...11.25
11.25...11.5
11.5...11.75
11.75...12
12...12.25
12.25...12.5
12.5...12.75
12.75...13
13...13.25
13.25...13.5
13.5...13.75
13.75...14
14...14.25
14.25...14.5
14.5...14.75
14.75...15
15...15.25
15.25...15.5
15.5...15.75
15.75...16
16...16.25
16.25...16.5
16.5...16.75
16.75...17
>17
14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S

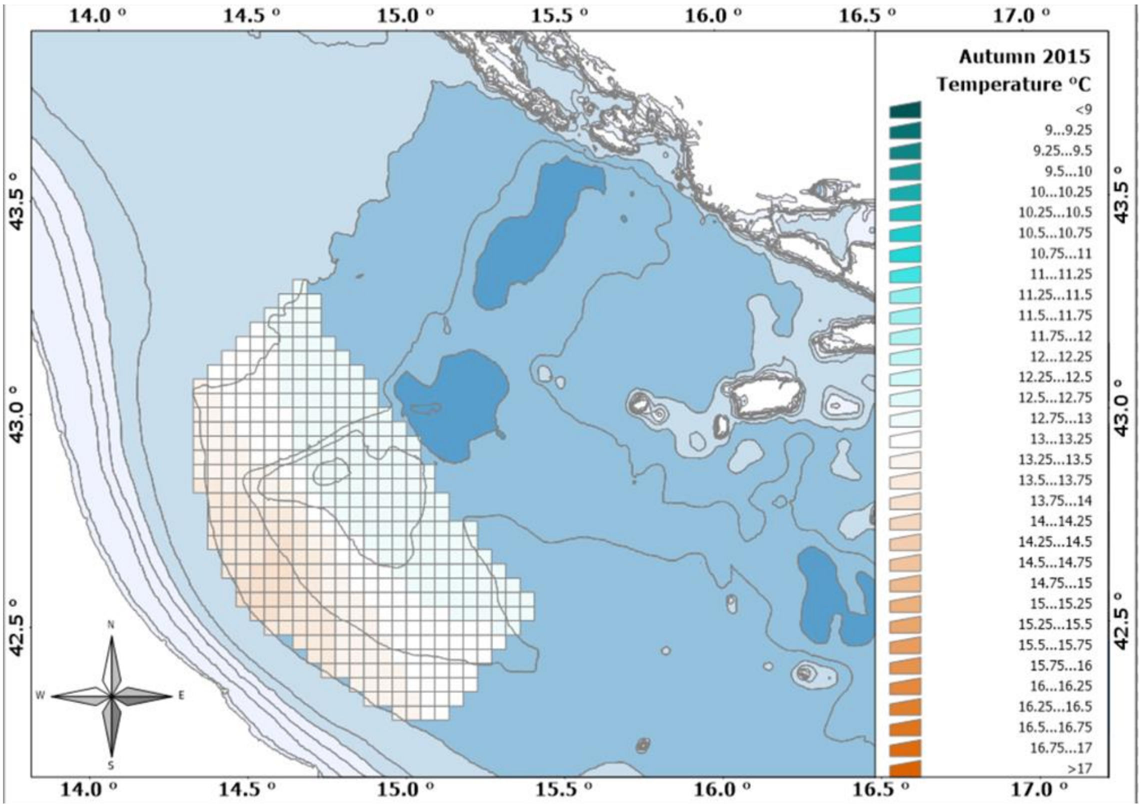

Spring 2015
Temperature °C
<9
9...9.25
9.25...9.5
9.5...10
10...10.25
10.25...10.5
10.5...10.75
10.75...11
11...11.25
11.25...11.5
11.5...11.75
11.75...12
12...12.25
12.25...12.5
12.5...12.75
12.75...13
13...13.25
13.25...13.5
13.5...13.75
13.75...14
14...14.25
14.25...14.5
14.5...14.75
14.75...15
15...15.25
15.25...15.5
15.5...15.75
15.75...16
16...16.25
16.25...16.5
16.5...16.75
16.75...17
>17
14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S

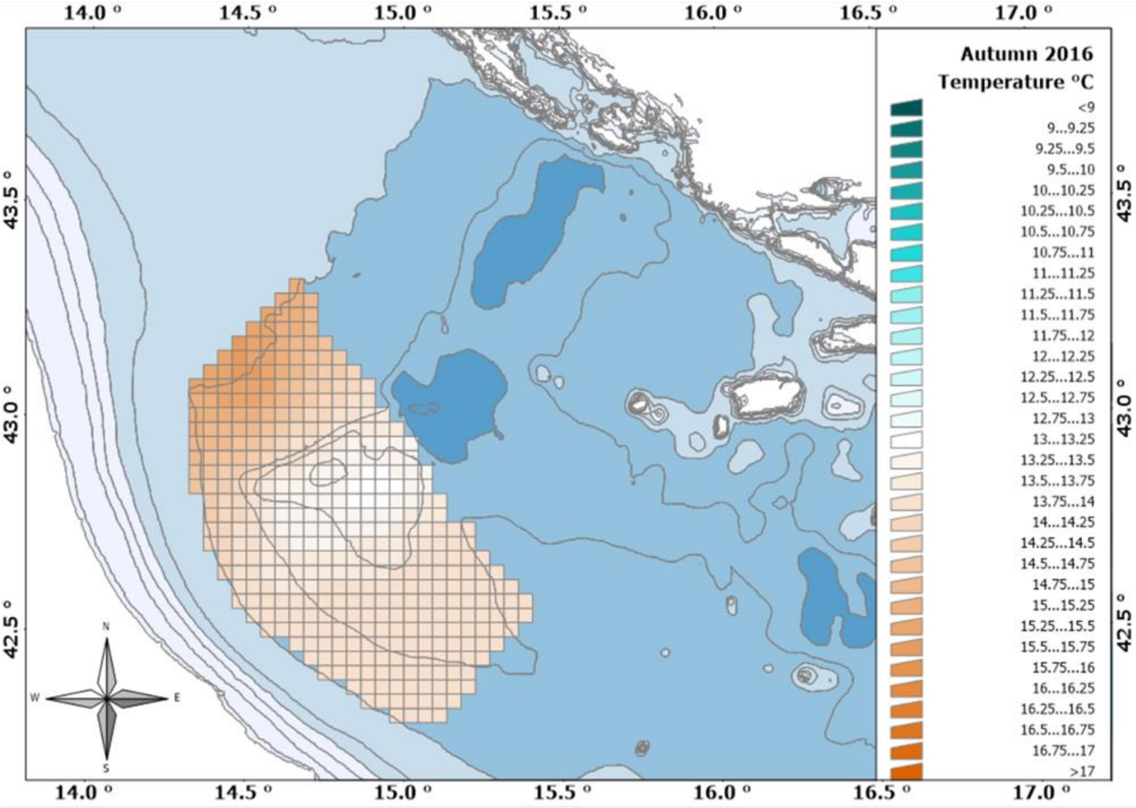

Spring 2016
Temperature °C
<9
9...9.25
9.25...9.5
9.5...10
10...10.25
10.25...10.5
10.5...10.75
10.75...11
11...11.25
11.25...11.5
11.5...11.75
11.75...12
12...12.25
12.25...12.5
12.5...12.75
12.75...13
13...13.25
13.25...13.5
13.5...13.75
13.75...14
14...14.25
14.25...14.5
14.5...14.75
14.75...15
15...15.25
15.25...15.5
15.5...15.75
15.75...16
16...16.25
16.25...16.5
16.5...16.75
16.75...17
>17
14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S

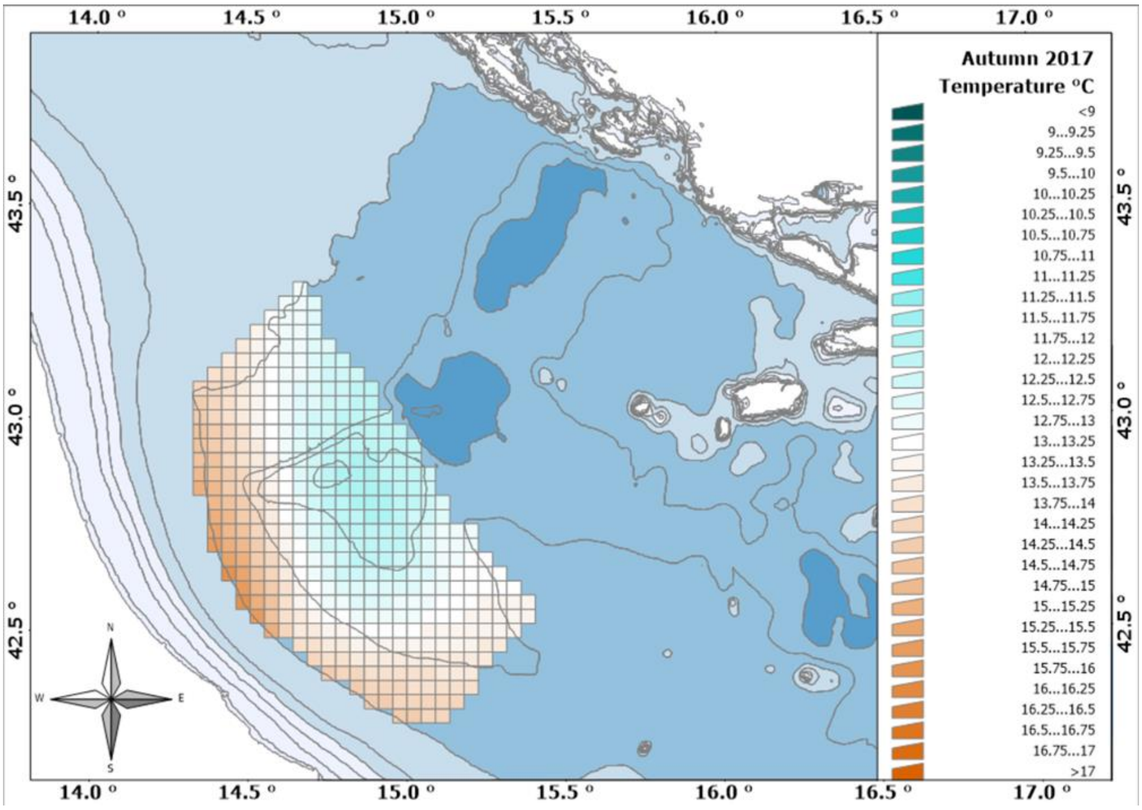

Spring 2017
Temperature °C
<9
9...9.25
9.25...9.5
9.5...10
10...10.25
10.25...10.5
10.5...10.75
10.75...11
11...11.25
11.25...11.5
11.5...11.75
11.75...12
12...12.25
12.25...12.5
12.5...12.75
12.75...13
13...13.25
13.25...13.5
13.5...13.75
13.75...14
14...14.25
14.25...14.5
14.5...14.75
14.75...15
15...15.25
15.25...15.5
15.5...15.75
15.75...16
16...16.25
16.25...16.5
16.5...16.75
16.75...17
>17
14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S

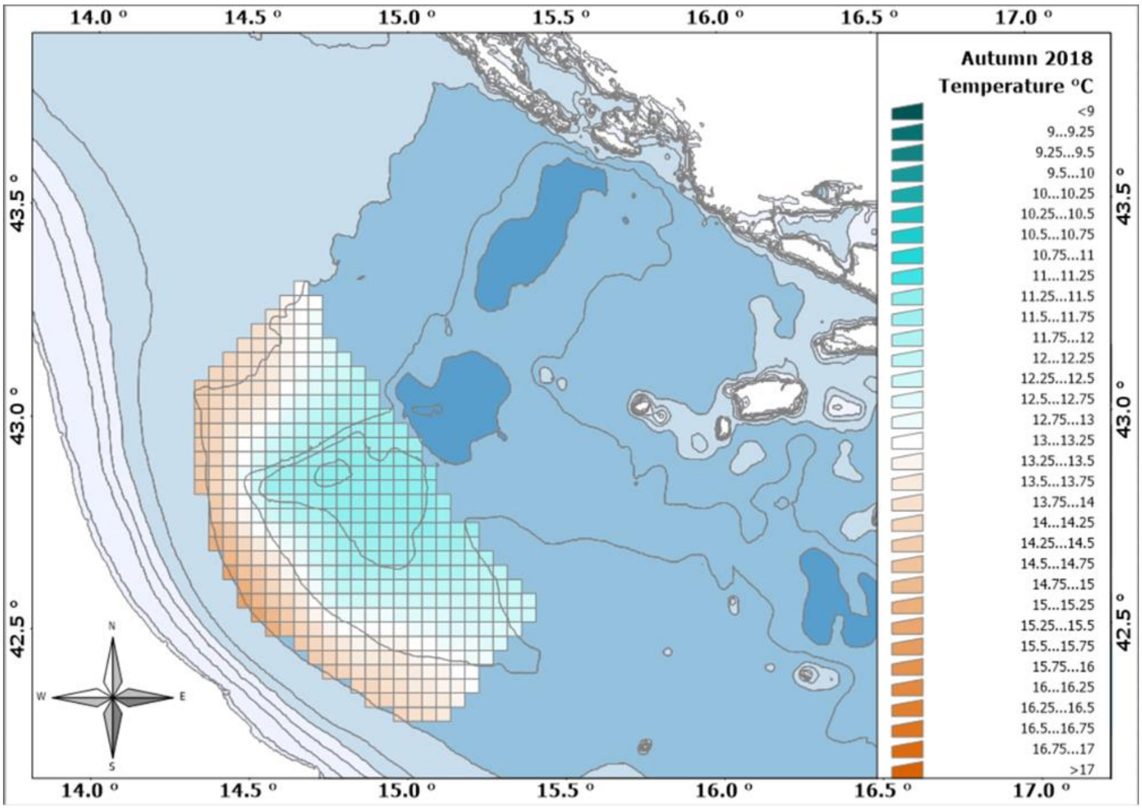

Spring 2019
Temperature °C
<9
9...9.25
9.25...9.5
9.5...10
10...10.25
10.25...10.5
10.5...10.75
10.75...11
11...11.25
11.25...11.5
11.5...11.75
11.75...12
12...12.25
12.25...12.5
12.5...12.75
12.75...13
13...13.25
13.25...13.5
13.5...13.75
13.75...14
14...14.25
14.25...14.5
14.5...14.75
14.75...15
15...15.25
15.25...15.5
15.5...15.75
15.75...16
16...16.25
16.25...16.5
16.5...16.75
16.75...17
>17
14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
N
W
E
S

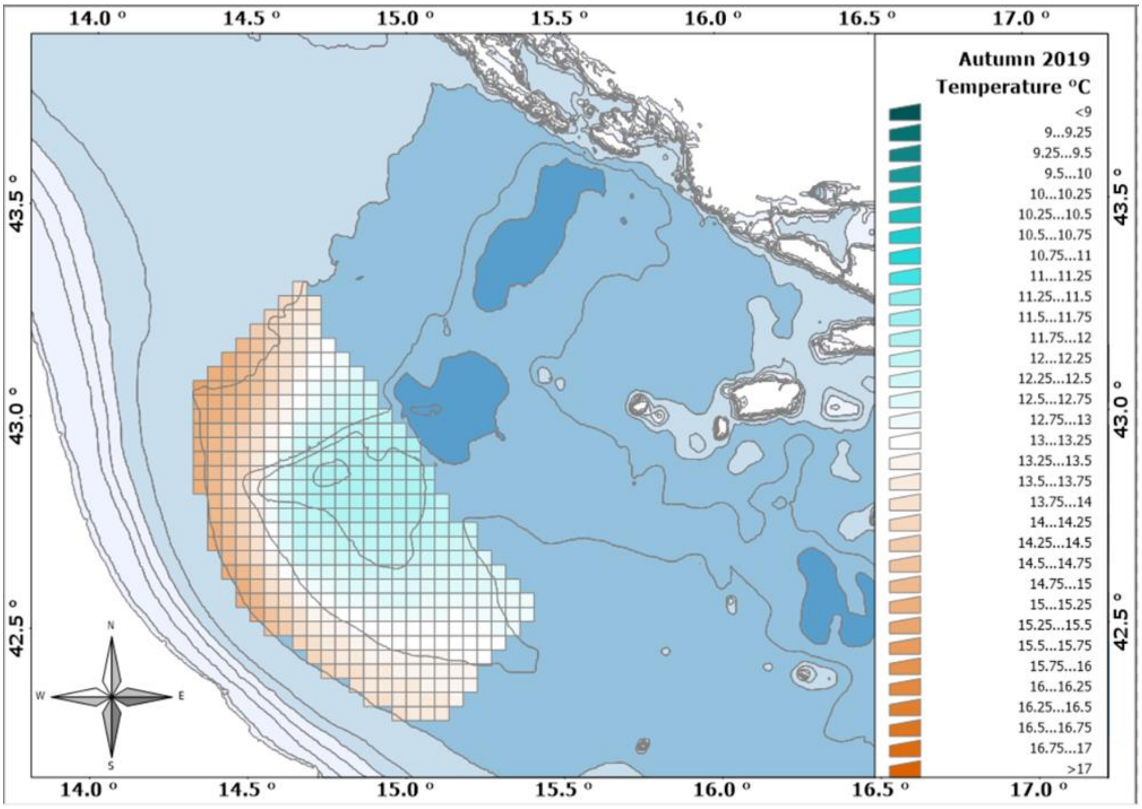
S11 Fig. Maps of bottom temperature values for the autumn time series. Bathymetry layer source: [75].






14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
Autumn 2017
Temperature °C
<9
9...9.25
9.25...9.5
9.5...10
10...10.25
10.25...10.5
10.5...10.75
10.75...11
11...11.25
11.25...11.5
11.5...11.75
11.75...12
12...12.25
12.25...12.5
12.5...12.75
12.75...13
13...13.25
13.25...13.5
13.5...13.75
13.75...14
14...14.25
14.25...14.5
14.5...14.75
14.75...15
15...15.25
15.25...15.5
15.5...15.75
15.75...16
16...16.25
16.25...16.5
16.5...16.75
16.75...17
>17
N
W
E
S


14.0 °
14.5 °
15.0 °
15.5 °
16.0 °
16.5 °
17.0 °
43.5 °
43.0 °
42.5 °
Autumn 2018
Temperature °C
<9
9...9.25
9.25...9.5
9.5...10
10...10.25
10.25...10.5
10.5...10.75
10.75...11
11...11.25
11.25...11.5
11.5...11.75
11.75...12
12...12.25
12.25...12.5
12.5...12.75
12.75...13
13...13.25
13.25...13.5
13.5...13.75
13.75...14
14...14.25
14.25...14.5
14.5...14.75
14.75...15
15...15.25
15.25...15.5
15.5...15.75
15.75...16
16...16.25
16.25...16.5
16.5...16.75
16.75...17
>17
N
W
E
S



Autumn 2019
Temperature °C
<9
9...9.25
9.25...9.5
9.5...10
10...10.25
10.25...10.5
10.5...10.75
10.75...11
11...11.25
11.25...11.5
11.5...11.75
11.75...12
12...12.25
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13...13.25
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16...16.25
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16.5...16.75
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14.0 °
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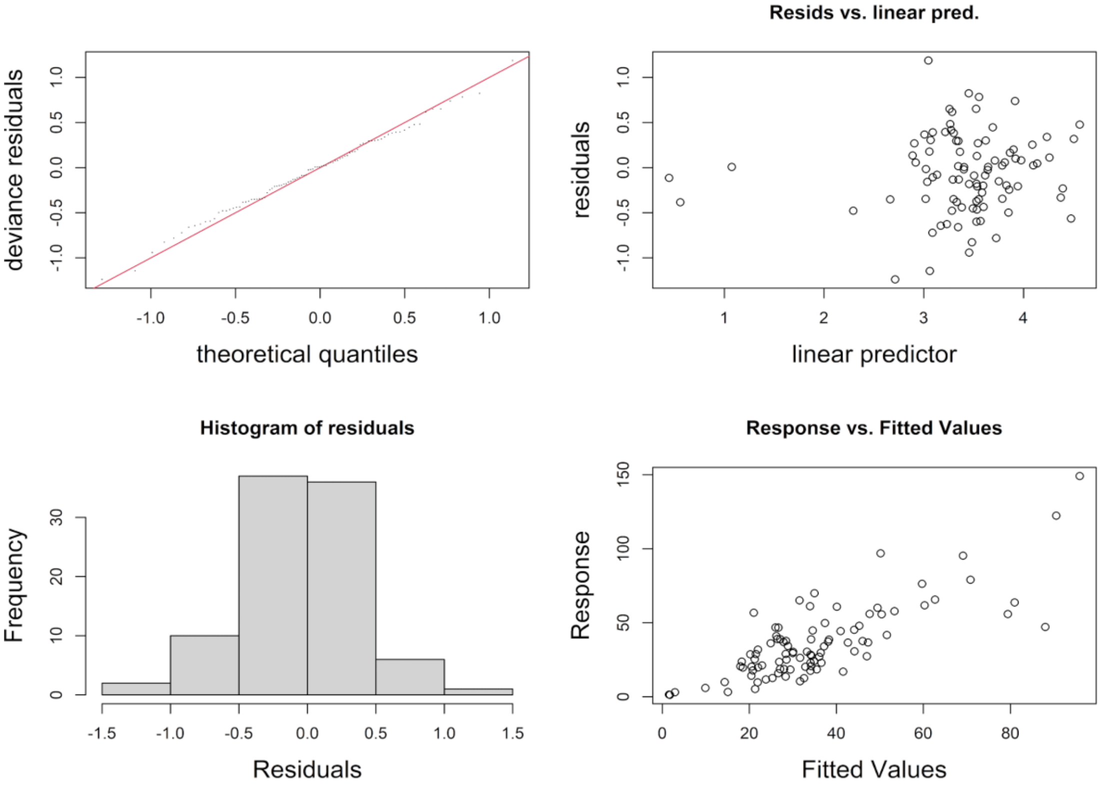
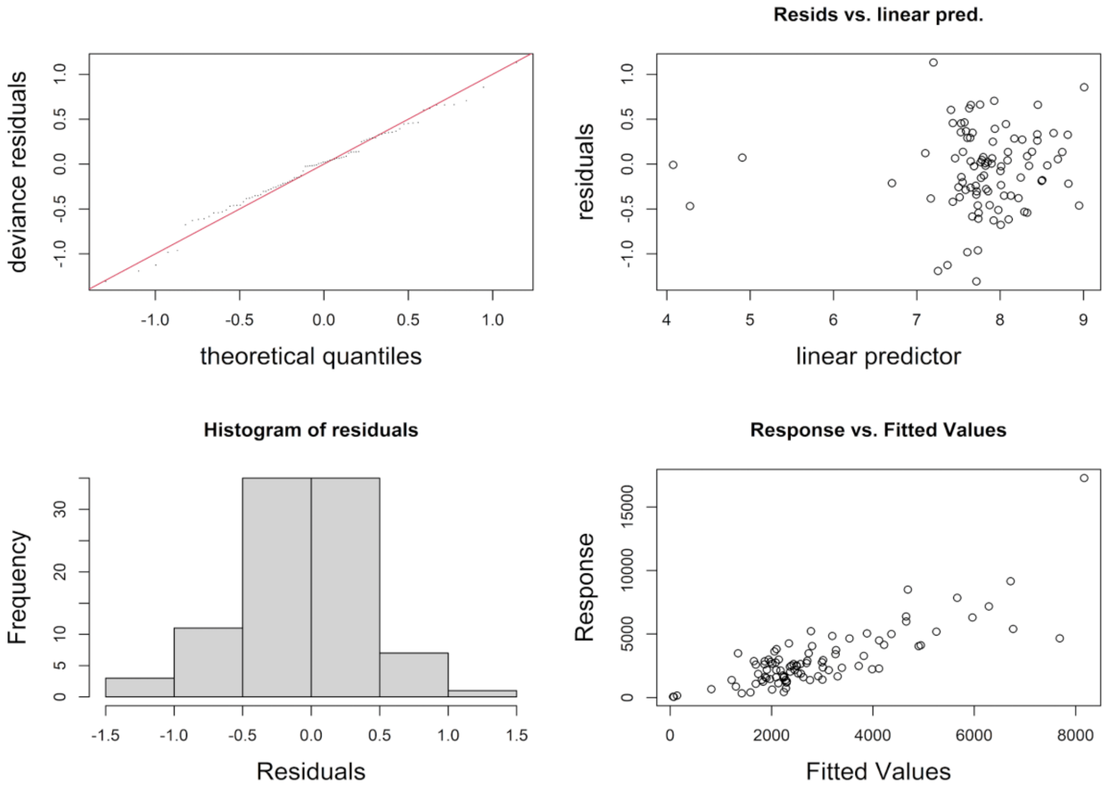
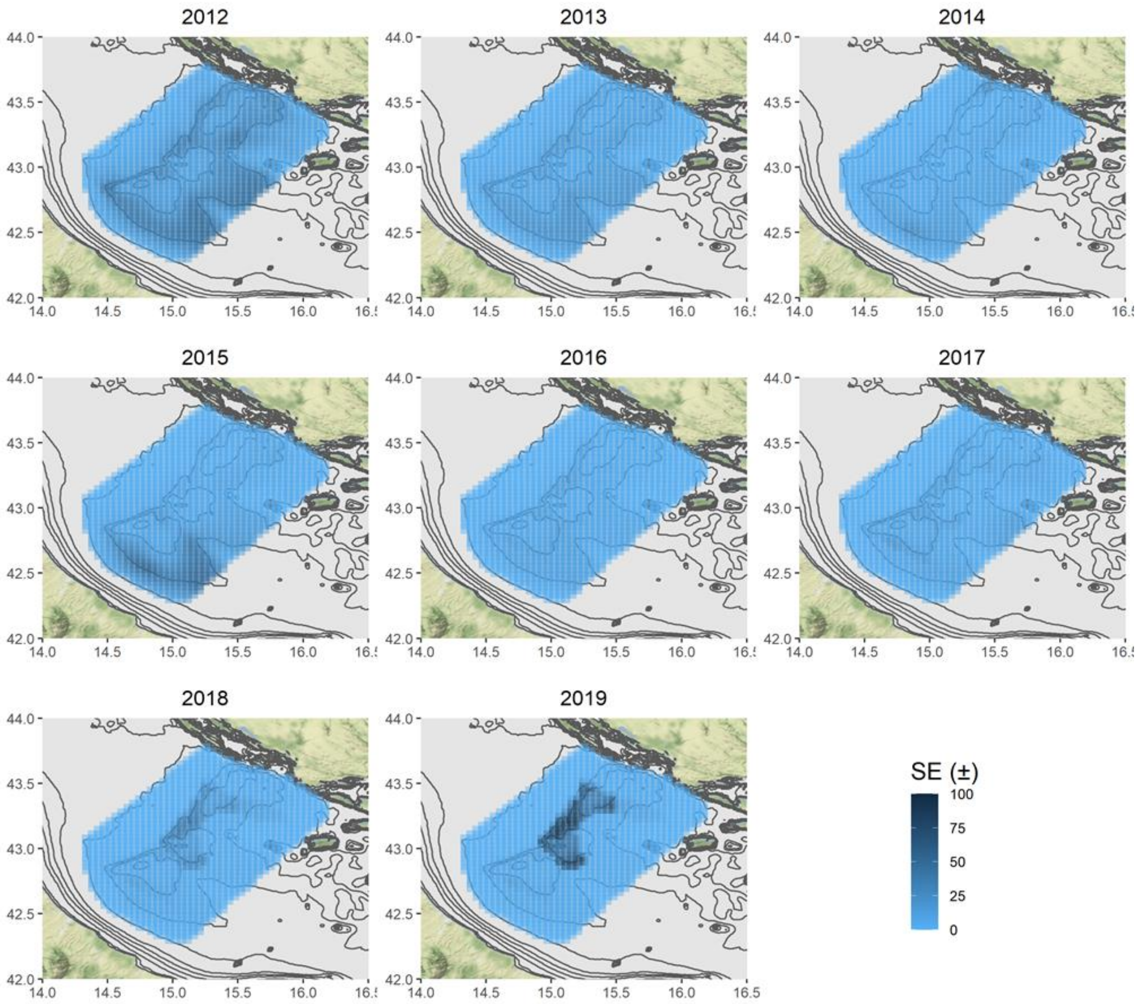


deviance residuals
theoretical quantiles
Resids vs. linear pred.
residuals
linear predictor
Histogram of residuals
Frequency
Residuals
Response vs. Fitted Values
Response
Fitted Values
-1.0
-0.5
0.0
0.5
1.0
1
2
3
4
-1.5
1.5
0
10
20
30
40
50
60
80
100
150

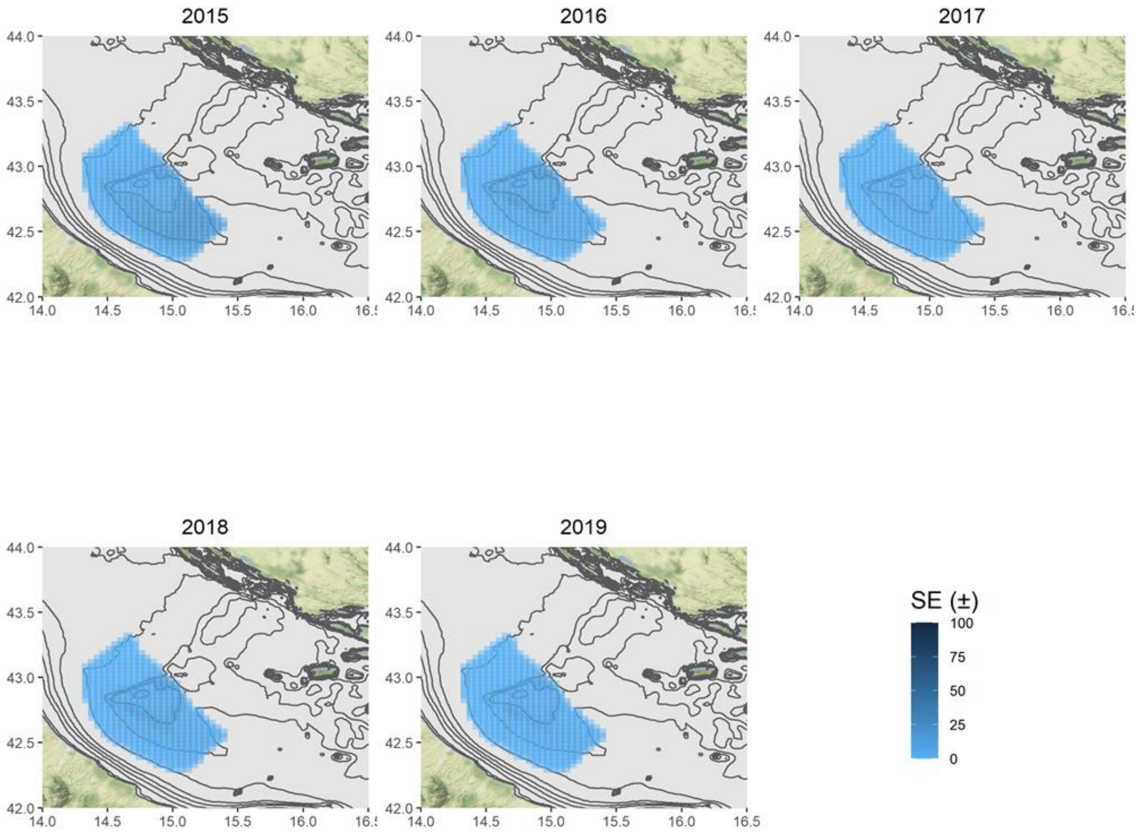
S12 Fig. Residual analysis for the final GAM for biomass index. Q-q plot, histogram and dispersion of residuals show mean around zero and homogeneity of variance.



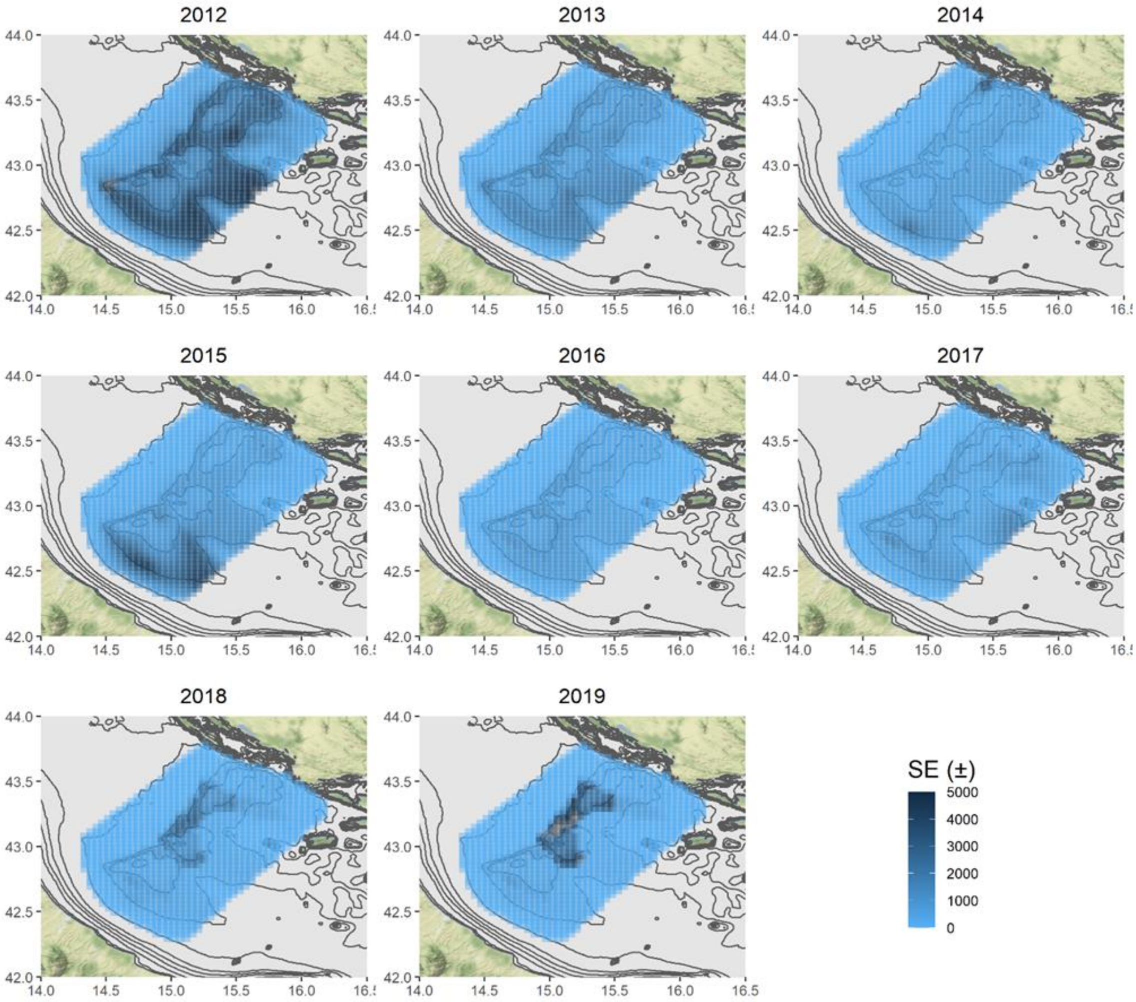
S13 Fig. Residual analysis for the final GAM for density index. Q-q plot, histogram and dispersion of residuals show mean around zero and homogeneity of variance.



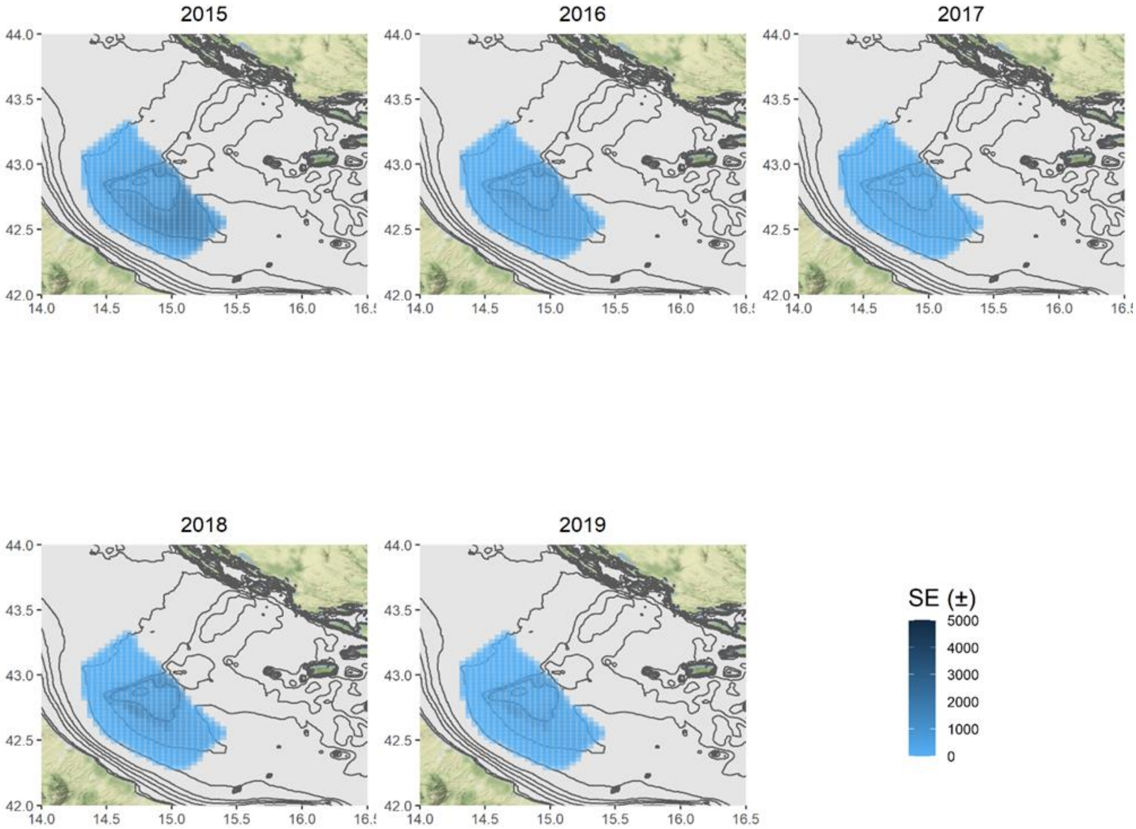
S14 Fig. Predicted standard errors of *Nephrops* biomass index (kg/km^2) for the spring time series. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.



S15 Fig. Predicted standard errors of *Nephrops* biomass index (kg/km^2) for the autumn time series. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.



S16 Fig. Predicted standard errors of *Nephrops* density index (N/km^2) for the spring time series. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.



S17 Fig. Predicted standard errors of *Nephrops* density index (N/km^2) for the autumn time series. Maps were made using the ggmap package [105] for R. Bathymetry layer source: [75]. Map tiles by Stamen Design, under CC BY Data by OpenStreetMap, under ODbL.

5. Assessing the state of *N. norvegicus* inhabiting the Pomo/Jabuka Pits

Currently, the assessment and management of Adriatic Sea fishery resources are based on data that do not fully account for the complex spatial patterns arising from fleet activity and/or species' behaviour and biology (Carpi et al., 2017; Russo et al., 2018). In the Mediterranean Sea, resources are managed at the Geographical Sub-Area (GSA) scale (Cataudella and Spagnolo, 2011). The definition of GSAs is based on management convenience rather than biological inference (Stephenson, 1999; Smedbol and Stephenson, 2001). Actually, the Adriatic Sea is divided into two management areas (GSA 17 and GSA 18) and official fishery-dependent data (i.e. landings) are delivered at this spatial scale. The FAO General Fisheries Commission for the Mediterranean (GFCM) and the EU Scientific, Technical and Economic Committee for Fisheries (STECF) already recognized the limits of a GSA-based approach stressing the need to appropriately account for complex patterns of fleet activity and/or different aspects of species behaviour and biology (STECF, 2014). At present, an alternative has not yet been found and fleets operate without considering the spatial structure defined by the FAO-GFCM GSAs, which instead represent the basis for stock assessment. With the aim to build a population dynamics model for *Nephrops* through a stock assessment indirect method (length-based model), the spatial domain of the study area was first defined. As described in the previous chapters, the Pomo/Jabuka Pits located along the Pescara-Šibenik junction in the Central Adriatic Sea represent an area of scientific and commercial interest characterised by a population of small-sized *Nephrops* individuals showing slow growth rates and small size at first maturity (Gramitto and Froglia, 1981; Froglia and Gramitto, 1988) if compared to other populations inhabiting Adriatic Sea (Froglia and Gramitto, 1988, Vrgoč, 2004). These differences in the biological traits pointed out the occurrence of a subpopulation of individuals living in the Pomo-Jabuka Pit area (Melaku Canu et al., 2020; Angelini et al., 2020). The "Pomo/Jabuka Pits" study area, hereafter referred as "Pomo", was therefore defined following ecological knowledge (Marini et al., 2016) and taking into account the locally implemented management measures (i.e. measures implemented from 2015 onwards; chapter 4.1); hence the study area includes the FRA zones plus an additional buffer zone that approximately follows the 100 meters bathymetry (northern and southern of the Pits; Figure 5.1).

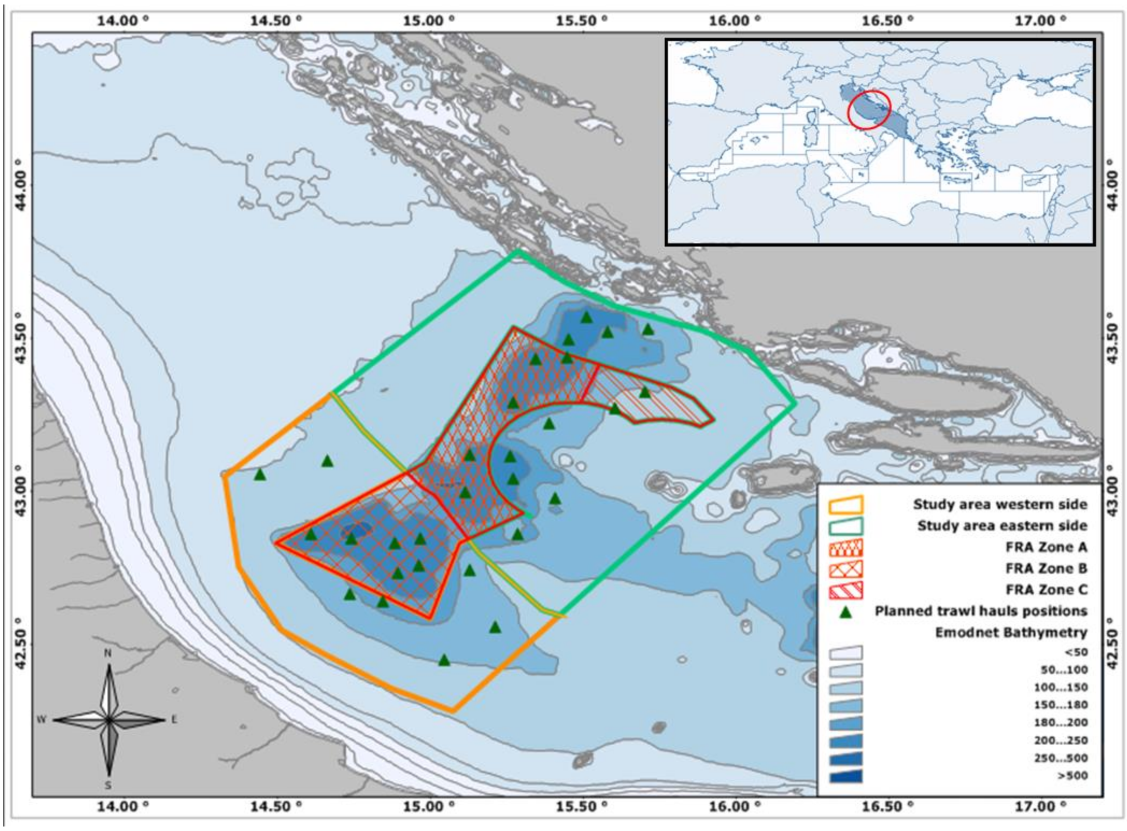


Figure 5.1. The study area. In the up-right rectangle the position of the study area within the Mediterranean basis is highlighted (red circle). The main map shows: the study area defined as "Pomo" divided in western side (orange polygon) and easter side (green polygon) in respect to the Adriatic midline. The FRA (red polygons) is based on the last GFCM recommendation (GFCM, 2017) and is thus divided into 3 zones with different management measures: zone A, B and C.

The characteristic sandy-muddy bottom of "Pomo" is ideal for the settlement of burrowing crustaceans as *Nephrops*, which is fished mainly on the seabed between almost 100 and 200 m of depth (chapter 4.2); the relatively high depth and distance from shores of the Pits excludes the use of gears such as traps (used mainly in channel areas of the northern Adriatic) and making bottom trawl nets the main fishing gear (Morello et al., 2009). The trawl fishery is regulated at an EU level by means of minimum landings size (MLS; 2 cm carapace length; 7 cm total length), mesh size (40 mm side square mesh; 50 mm side diamond mesh) and by promoting the development of management plans for trawling and other fishing activities within the territorial waters (EC regulation 1967/2006). In this context, seasonal fishing closures (usually 40 days per year in summer) regarding Italian bottom and mid-water trawl fisheries were adopted (Demestre et al., 2008). The main actors in the trawl fishery for *Nephrops* in the Adriatic are Italy and Croatia, with Italy fetching by far the highest catches since the 1970's (FAO, 2011–2017). The contribution of Croatia to total Adriatic landings, on average, accounts for 28% in weight with an average landing of about 315.4 tons from

1992 to 2018 (FAO, 2021). The trawl fishery for *Nephrops* in the Adriatic Sea is characterised by fluctuating landings throughout the years, with peaks at about 2000 tons in 1992 and 2005 (only for Italy) and decreasing in more recent years (Figure 5.2).

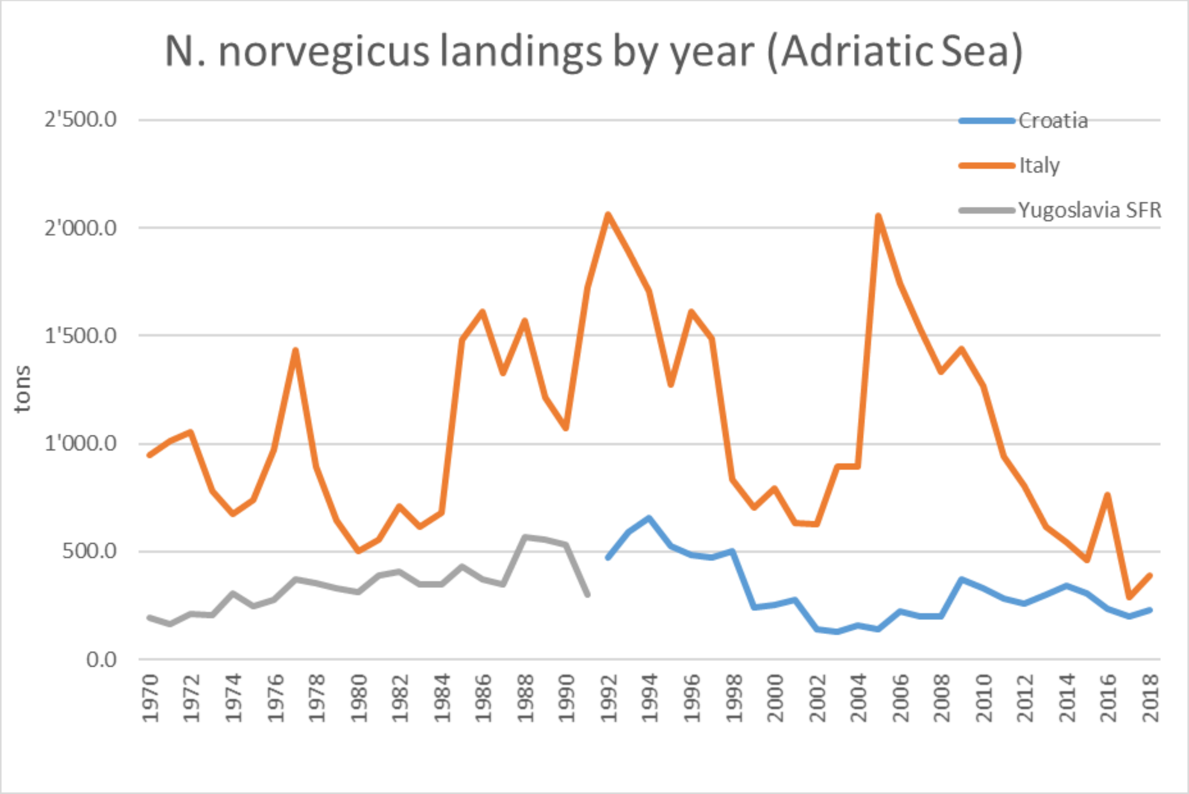


Figure 5.2. Annual Adriatic landings (FAO area 37.2.1) for *Nephrops norvegicus* divided by country (Italy, Croatia and ex-Yugoslavia) from 1970 to 2018. (FAO, 2021)

Nephrops' catches during the year are not constant but show seasonal peaks, such oscillations are mainly due to the behaviour of this species which alters the rates of emergence from the burrows (Wieckzorek et al., 2001; Atkinson and Eastman, 2015; Sbragaglia et al., 2017; chapter 1.3). Since individuals can only be caught when they are outside or in the proximity of the burrow's entrance, this pattern of rhythmicity should be considered in the assessment of the exploitation of a stock as a factor affecting the availability of the resource (i.e. catchability; Aguzzi et al., 2004a). Seasonal emergence patterns are generally characterised by poor emergence rates for both sexes during the winter season (Marrs et al., 2000) and a maximum corresponding to the reproductive season (which is observed in the Adriatic during late spring and early summer; Gramitto and Froglia, 1981); typically females does not leave their burrows during the egg-bearing period (Bell et al., 2006), while juveniles tend to spend more time in their burrows, especially during the first year of life (Powell & Eriksson, 2013). To deal with the sex-ratio proportion in the catches during the year, within this study it was decided to arbitrarily divide the solar year into 3 different time steps (TS):

- TS 1 = from January to March (male emergence is dominant)
- TS 2 = from April to July (both sex emerge equally)
- TS 3 = from August to December (male emergence is dominant)

The choice of such a TS division was made following the biological and environmental knowledge of *Nephrops* emergence patterns in the Adriatic Sea. Specifically, some recent local studies showed that ovarian maturation starts in April and reaches a peak in June/July, while brooding period shows maximum values in November, leading to a shift towards males in the sex ratio proportion (Colella et al., 2012; Angelini et al., 2020). The results of these studies are in accordance with previous literature about seasonal catchability of the species in the Adriatic Sea (Froglia and Gramitto, 1981) as well as in the Western Mediterranean (Aguzzi et al., 2004b, 2007). These studies also identified the maximum vulnerability period for both sexes; it was thus observed that the period of maximum emergence of *Nephrops* for both sexes was approximately from April/May to June/July. Furthermore, observations on the sex-ratio reported in catches from seasonal local surveys within "Pomo" showed a mean sex ratio (males/males+females) of about 0.41 during the early spring period (April/May) and of about 0.58 during the autumn season (September/October/November; Martinelli et al., 2021). Official commercial data are provided annually at a month level, but commercial sampling data are not always available at this temporal dimension (Russo et al., 2018). From a biological point of view, TS3 could be aggregated with TS1 of the following year, because they are equivalent, but to simplify data treatment, within this study it was decided to follow the solar year temporal domain and keep 3 TS within each year. All input data presented below were therefore split according to the seasonal emergence rates at the TS level.

The main sources of fisheries-dependent data for the Adriatic come from the National Programs implemented within the Data Collection Framework (DCF), that since 2000 collects fisheries data to support the Common Fisheries Policy (CFP) through scientific advice (EU regulation 2017/1004). Fishery-dependent data comprise different types of data collected by national DCF programs: (i) official landings and discard data (recorded by observers onboard commercial bottom trawlers); (ii) length frequency distributions observed in commercial samples (LFDs); and (iii) biological information as sex-ratio and length-weight relationship. Data are usually reported at GSA level, thus it was necessary to split them to derive those related to the study area ("Pomo") and properly apply a length-based model. This was done in different ways according to the country involved and their method of collecting fishery-dependent data. In population dynamics modelling it is well known that length-based models require many assumptions and are specifically tailored to particular species and/or stocks (Smith and Addison, 2003). Therefore, the evaluation of the state of *Nephrops*' population presented in this chapter took into account most of the specificities of this species (see above) and the raw structure of the data sources. The simplest possible stock assessment models were developed with the aim of minimizing the number of assumptions and thus the resulting errors and uncertainties in the estimates.

5.1 Treatment of Italian data available through DCF for the period 2009-2019

For the Italian fishery-dependent data, the monthly landings for the selected metier (i.e. OTB_DES_>=40_0_0 corresponding to otter board trawlers with a Length Overall > 40 m targeting demersal species) for GSA 17 and 18 were made available by Mably analysis and research center (http://www.mably.it/). As stated in the introduction section, in Mediterranen waters Nephrops are caught mainly by bottom trawlers; however, an assumption about the type of fishing gear/metier adopted was mandatory cause other beam trawl (e.g. “rapido” trawl, et al., Colloca et al., 2015) or even traps was historically used to catch *Nephrops* (e.g. Morello et al., 2009). In the dataset presented here, only the metier OTB_DES_>=40 was selected because from 2008 onwards the differences between its landings and those from other metiers were considered negligible (Figure 5.3).

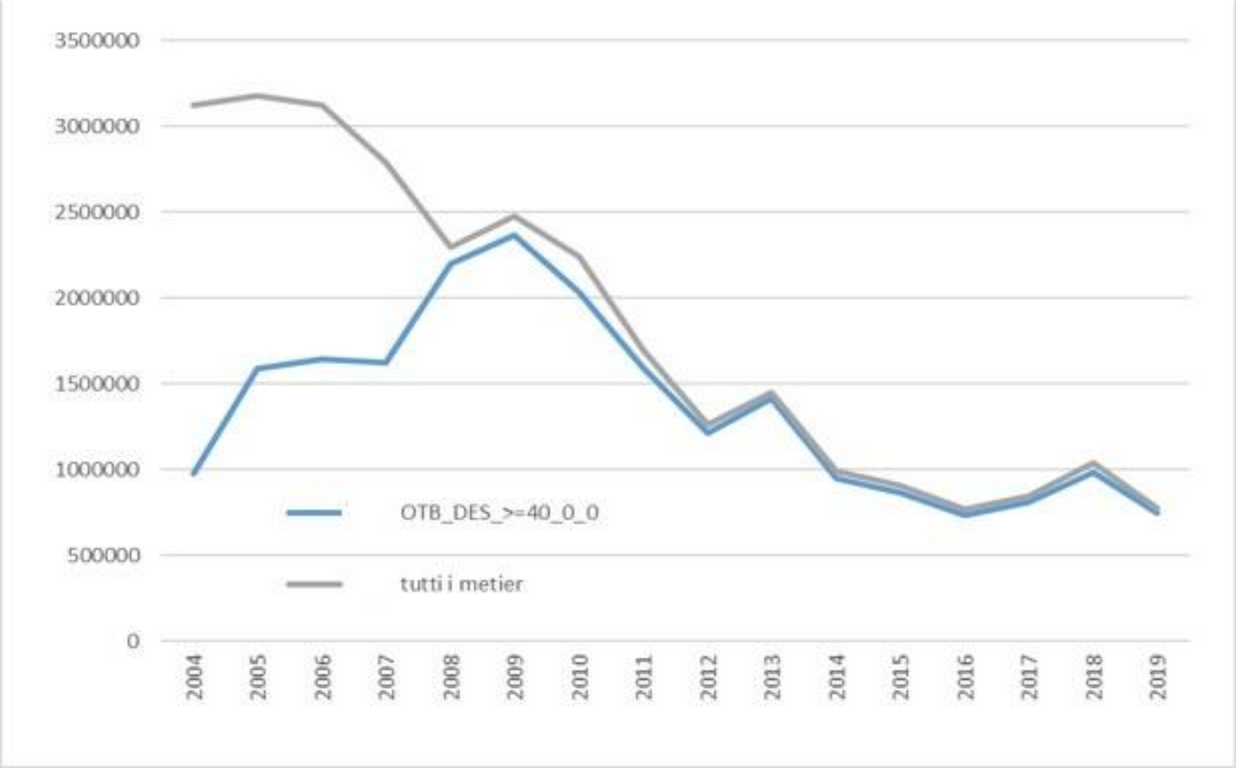


Figure 5.3. Comparison between the official italian landings (2004 - 2019) of all metiers (grey line) and the official italian landings filtered only for OTB_DES_>=40 (blue line). The landings on the y-axis are in kilograms.

A method for estimating the monthly landings of Italian trawlers operating in the Adriatic Sea at a higher spatial resolution than the GSA was developed by Russo et al. (2018); this was then applied to estimate the *Nephrops* production for the “Pomo” study area both in terms of landing’s origin (i.e. fishing grounds) and destination (i.e. harbours). This method combines questionnaire-derived vessel-specific landings with the spatial origin of the landings as reconstructed a posteriori using the Vessel Monitoring System (VMS). The VMS consists in a tracking device installed on board each fishing vessel with length overall (LOA) ≥ 15 m (EC, 2002; EU, 2011) that periodically sends data on vessel position and speed via satellite transmission. Through R packages such as VMSbase (Russo et al., 2014), these pings (provided by the VMS) were then managed, processed and visualized allowing to track the fishing vessels activity and accurate estimate the fishing effort directly associated with the logbook catches. Therefore, such an approach was used to estimate the amount of *Nephrops* Italian landings ascribable to the “Pomo” study area in the years for which reliable VMS data were available as well as monthly official Italian landings: 2009 – 2019. This is crucial especially for the “Pomo” area, for which VMS data revealed that fishing activity is made by boats originating from both GSA 17 and 18, landing their catches in their respective home harbours (Russo et al., 2018). Results revealed that the effort made by vessels from the GSA 18 fleets (according to the home/landing harbour) in GSA 17 (“Pomo” area) is higher than the effort made by vessels from GSA 17 in GSA 18. Hence, with the overarching goal to estimate the amount of landings ascribable to the “Pomo” study area, landings obtained for boats which actually fished in “Pomo” from both GSA 17 and 18 were aggregated by TS.

A preliminary analysis carried out on data collected within the periodical biological sampling of commercial catches (CAMPBIOL) conducted by CNR IRBIM in GSA 17 (as part of the Italian national plan for DCF), showed that for *Nephrops* fishery the discard rates are very low and thus could be considered negligible in the total catch and not taken into account for further analyses and elaborations. Figure 5.4 shows the Italian estimated landings from 2009 to 2019 for the “Pomo” study area obtained for each considered time step.

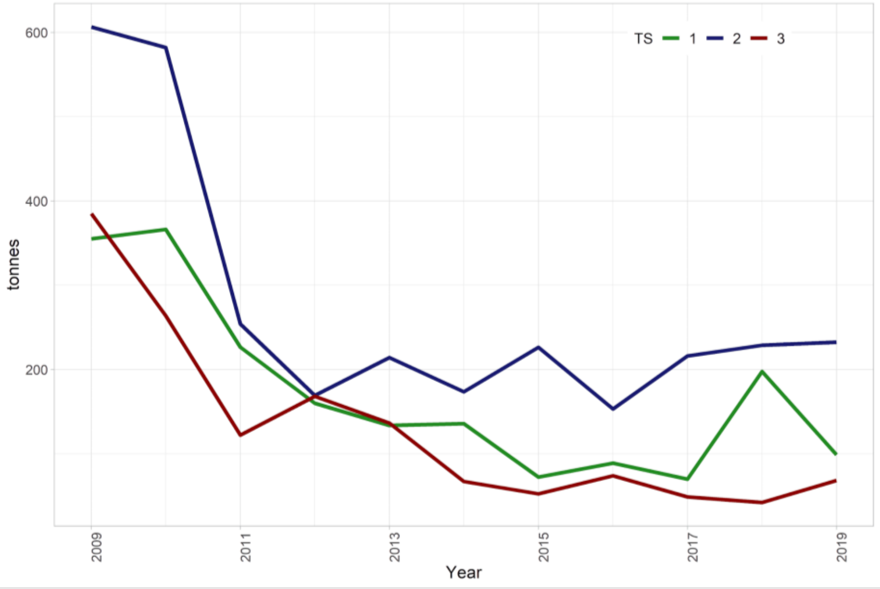


Figure 5.4. Italian landings (2009-2019) by time step for the "Pomo" area. The time steps are indicated with different colours.

Length composition of *Nephrops* Italian landings within "Pomo" were as well derived from CAMPBIOL. Data were collected by scientific observers onboard commercial bottom trawlers or on samples landed in the monitored harbours. CNR IRBIM observers in addition to the mandatory information required by the Italian National Program also collect on a voluntary basis georeferenced data allowing to discriminate the geographical origin of the examined sample within the GSA. The individuals were all measured for carapace length (CL) to obtain data about the size structure of the population; in addiction, when was possible, a subsample was collected to record individual weight, sex and maturity stage in order to obtain sex ratio, length-weight relationship, size at first maturity. The observed monthly length frequency distributions (LFDs) obtained only from samples collected in the "Pomo" area were raised to the previously obtained landings for TS in order to obtain numbers of individuals landed for each size class (1 mm CL). The Italian length compositions in "Pomo" were aggregated by TS, as previously done for landings estimates. The LFD data were then plotted by time step and year (Figure 5.5).

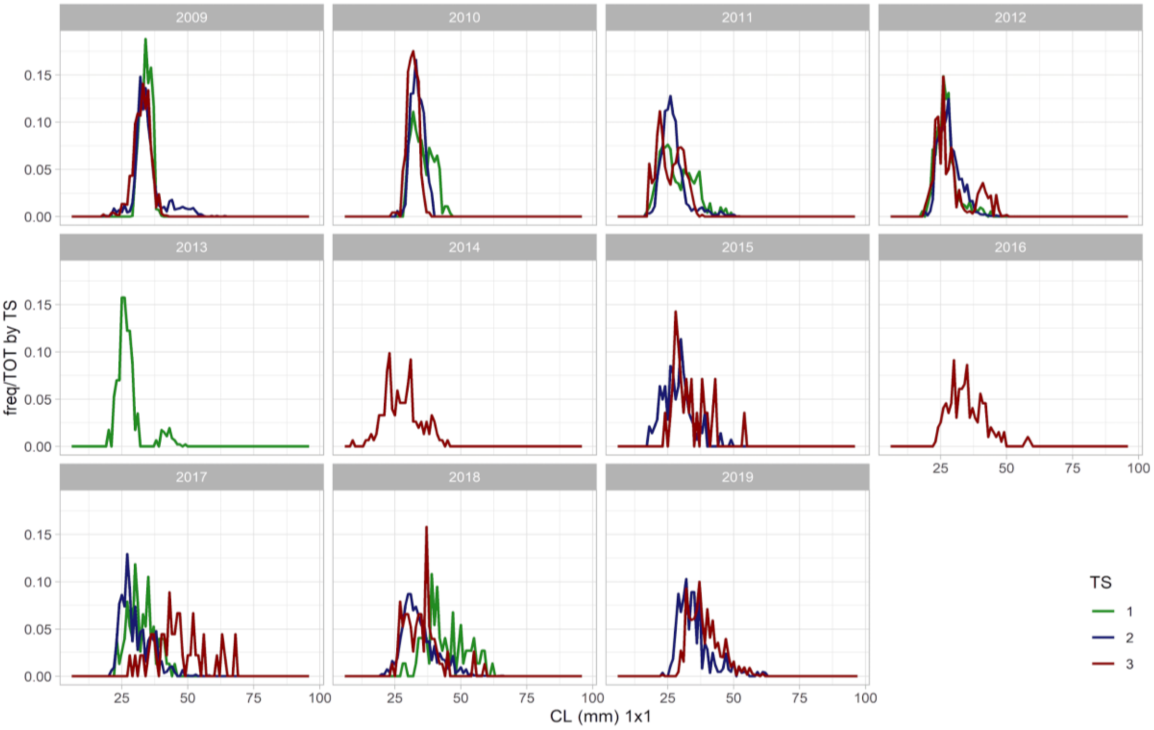


Figure 5.5. LFD (1 mm CL) for "Pomo" Italian landings (2009 - 2019) from CAMPBIOL raised at a TS level.

In order to check consistency with behavioural information on the seasonal emergence rate differing between sexes described above, an analysis on the sex-ratio proportion by year and month was also carried out on the data obtained from the biological subsamples. The sex-ratio (Males/(Males + Females)) was at first calculated by year and splitted by TS with the aim to investigate qualitatively the proportion between sexes over years (Figure 5.6). If repeated differences in sex proportion over the years were observed, the previously defined TS setting would have to be changed. Then, the data from 2009 to 2019 were aggregated by month and also splitted by TS (Figure 5.7).

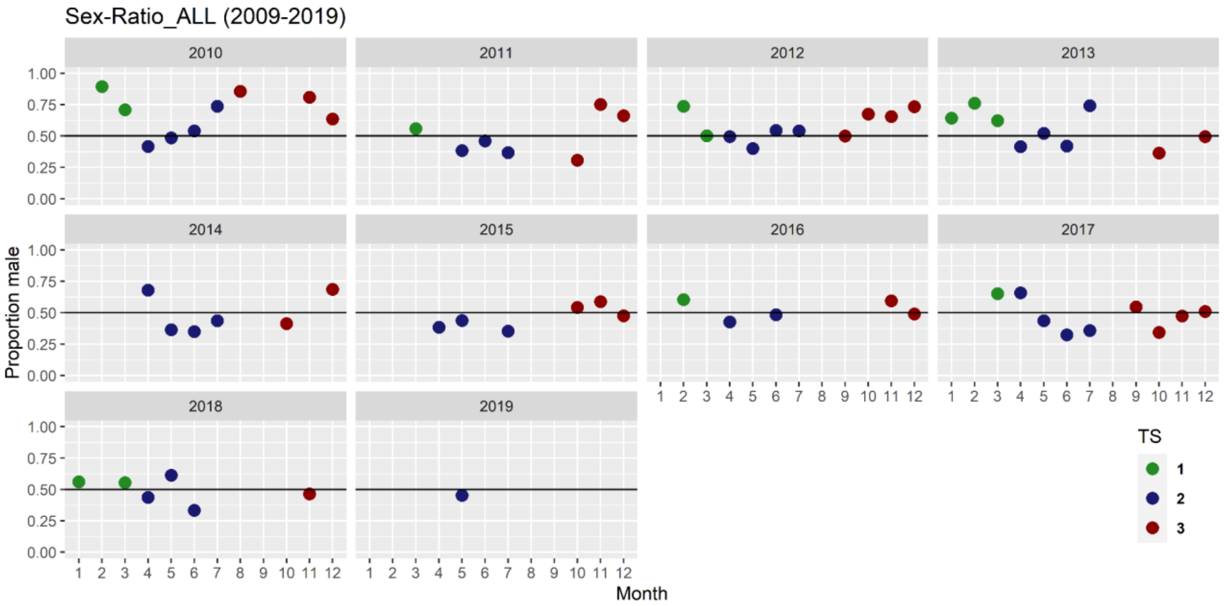


Figure 5.6. Italian sex-ratio proportion by TS and year (2009 - 2019). TS1 = Jan_March (green dots); TS2 = Apr_Jul (blue dots); TS3 = Aug_Dec (red dots). Data for 2009 were missing.

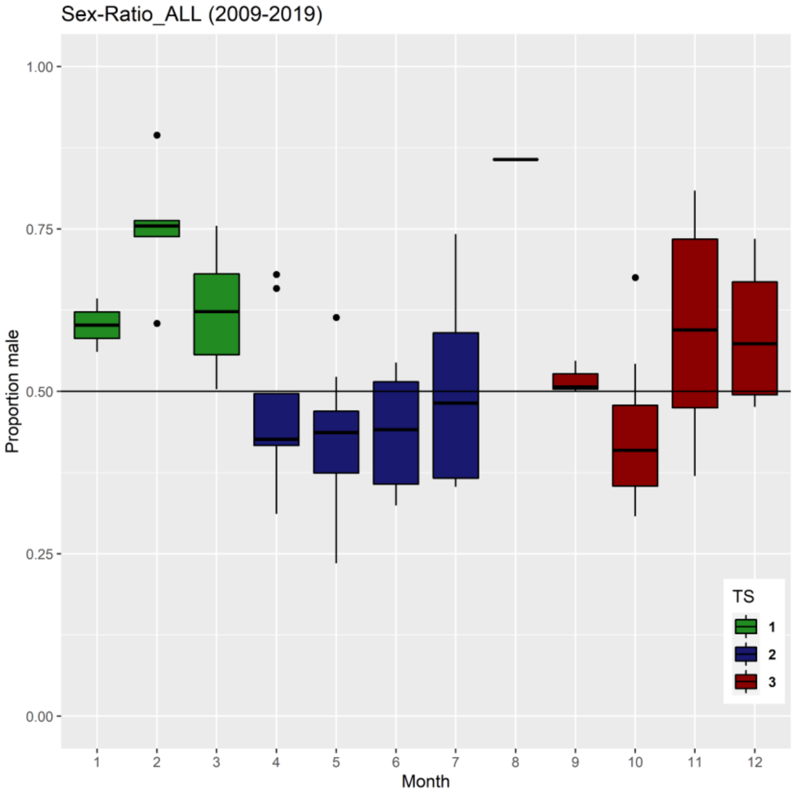


Figure 5.7. Boxplot of Italian sex-ratio proportion (Males/Males + Females) by month with indication of relative time step (TS)

The results showed a good correspondence between the sampled sex-ratio proportion and the TS pre-defined in accordance with the emergence rhythms of both sexes along the year. Furthermore, a smoothing square function weighted on the number of sampled individuals (Figure 5.8) was calculated. By means of smoothing functions fitted on biological LFDs data, the sampling variability among size classes and years can be reduced while maintaining the difference between TS. Indeed, biological sampling are not always equally balanced resulting in different sample size among size classes; it could lead to an incorrect sex-ratio proportion for missing or unrepresentative size classes (i.e. too small sample size). The analyses were carried out using the statistical software R ver. 3.5.2 (R Development Core Team, 2020). A cross validation was conducted in order to select the more confident number of degrees of freedom for each function. These functions were estimated to predict the sex-ratio by size classes to be applied to the “Pomo” Italian LFDs splitting each size classes by sex accordingly to TS partition (Figure 5.9). The size classes were then aggregated into 2 mm length categories (2x2) in order to be entered in the stock assessment model. The total number of commercial samples was adopted as “process error” (i.e. sample size) driving the weight given to the observed data in the subsequent model fitting process (Bull et al., 2012).

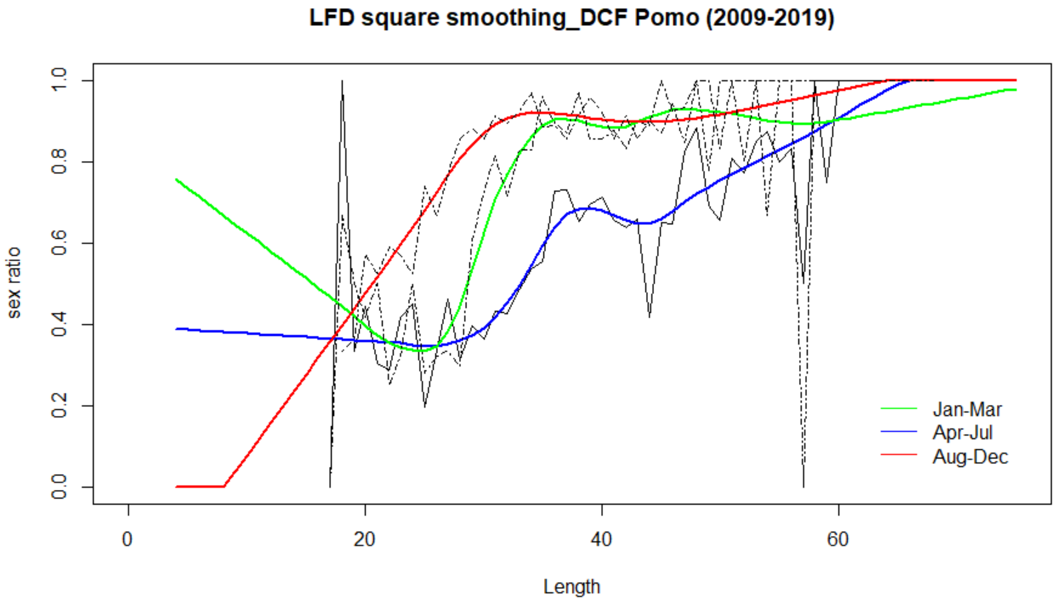
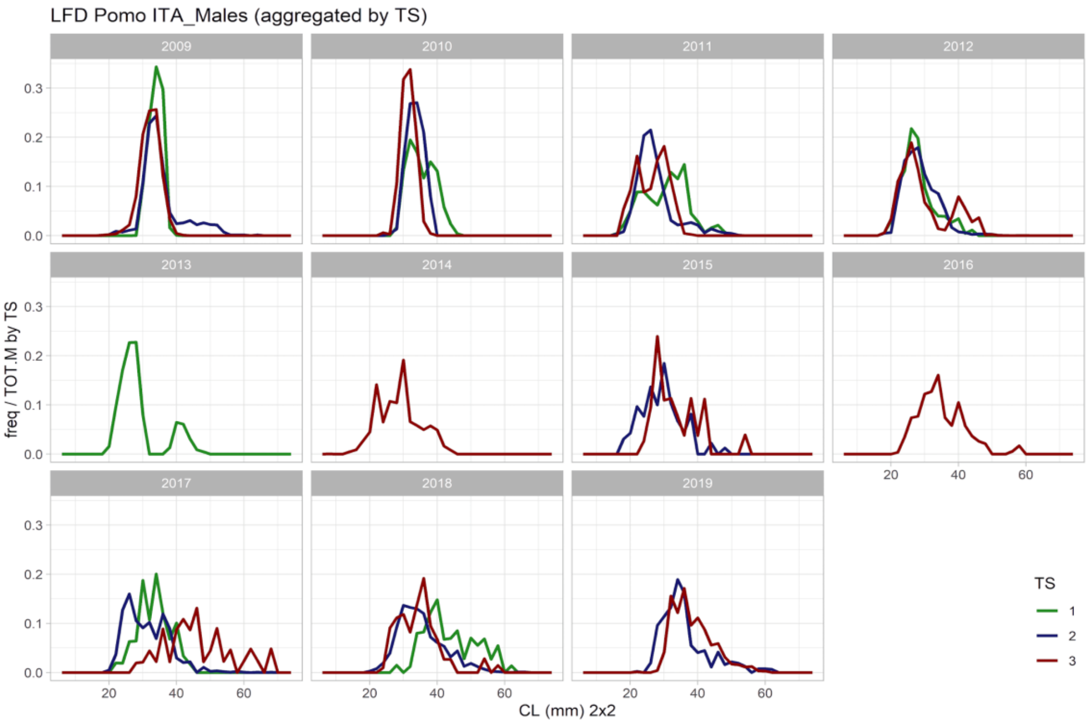


Figure 5.8. Sex ratio (Males/(Males + Females)) smoothing functions by time step from Italian DCF data. The coloured lines represent the smoothing functions for TS1, TS2 and TS3 (green, blue and red lines, respectively), the black lines show the calculated sex-ratio for each previously mentioned time step (dot, dash-dot and continue lines, respectively).

The observations on biological data confirm that the maximum peak of emergence for females is the TS2, when they come out from burrows to mate, while male’s emergence is dominant for the rest of the year. Such a rhythmicity is consistent for the larger size classes (>20 mm), while for the smaller size individuals, which are assumed not to have reached the sexual maturity, the proportion between the sexes is more balanced.



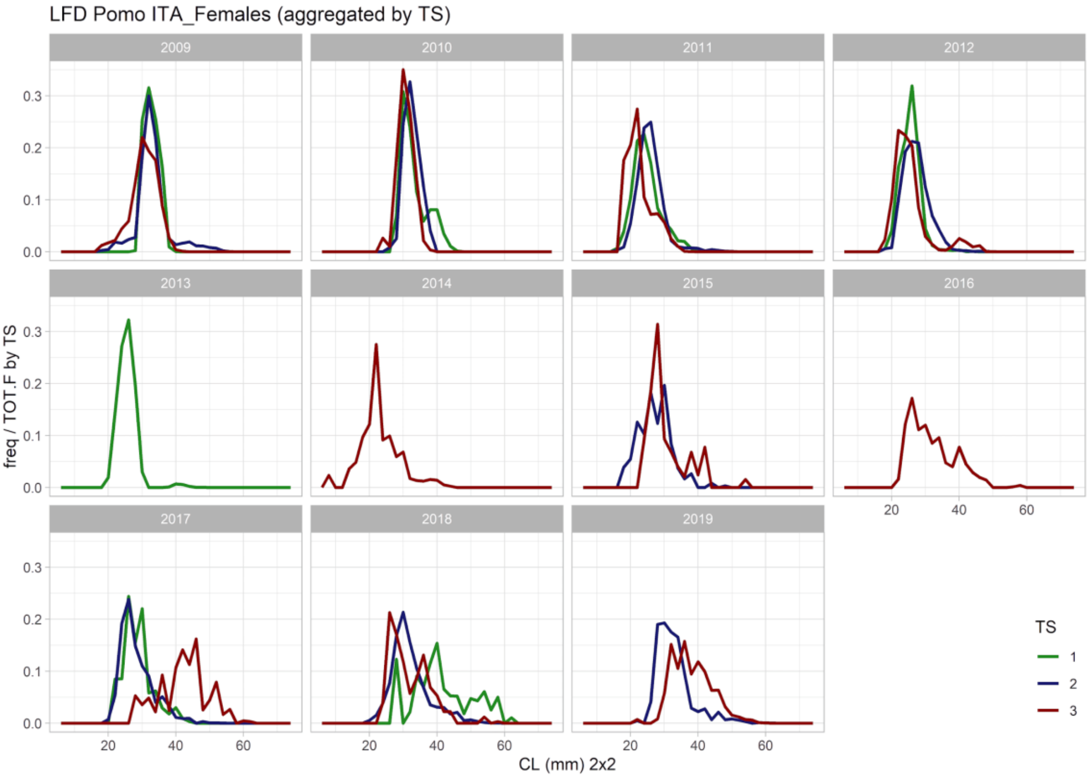


Figure 5.9. Italian “Pomo” LFD frequencies (2009 - 2019) by sex (upper panels are males, bottom panels are females) and time step (TS1 = Jan_March (green lines); TS2 = Apr_Jul (blue lines); TS3 = Aug_Dec (red lines)) from Italian DCF data. The CL (mm) were aggregated by 2x2 size classes.

5.2 Treatment of Croatian data available through DCF for the period 2009-2019

The Croatian fishery-dependent data were provided by the Institute of Fisheries (IOF) of Split (Croatia) for FPO and OTB fishing gears based on the Croatian DCF; where OTB means otter board trawling fleet and FPO includes creel fishing activities. A different landing proportion was observed for the two different fishing gears reporting an average proportion between FPO and OTB of less than 1/10 of the total Croatian landings (Figure 5.10). Hence, only the data from OTB were used due to the actual negligibility of collected landings by FPO and also to maintain the consistency with the previous assumption adopted for the Italian fishery-dependent data (chapter 5.1).

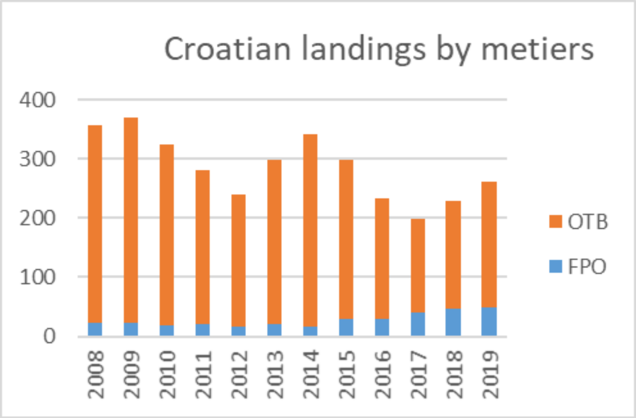


Figure 5.10. Tonnes of total annual Croatian landings by gear. “OTB” means otter board trawling fleet and “FPO” includes creel fishing activities.

As for the official Italian landings, the Croatian discard rates were very low, and these could be considered negligible in the total catch and were thus not taken into account. The *Nephrops* production estimates for OTB were then splitted by TS and spatial domain: indeed the “Pomo” adopted study area partially corresponds to the Croatian fishing grounds C1, C2, C3, C4, J1, J2, G3 adopted in the Croatian DCF sampling program (Figure 5.11). For the first years of the available Croatian time series (2008-2011) data were not provided by fishing grounds; hence, the catches from 2008 to 2011 were estimated through a catch ratio calculated on the first 3 available years (2012-2014) for each time step.

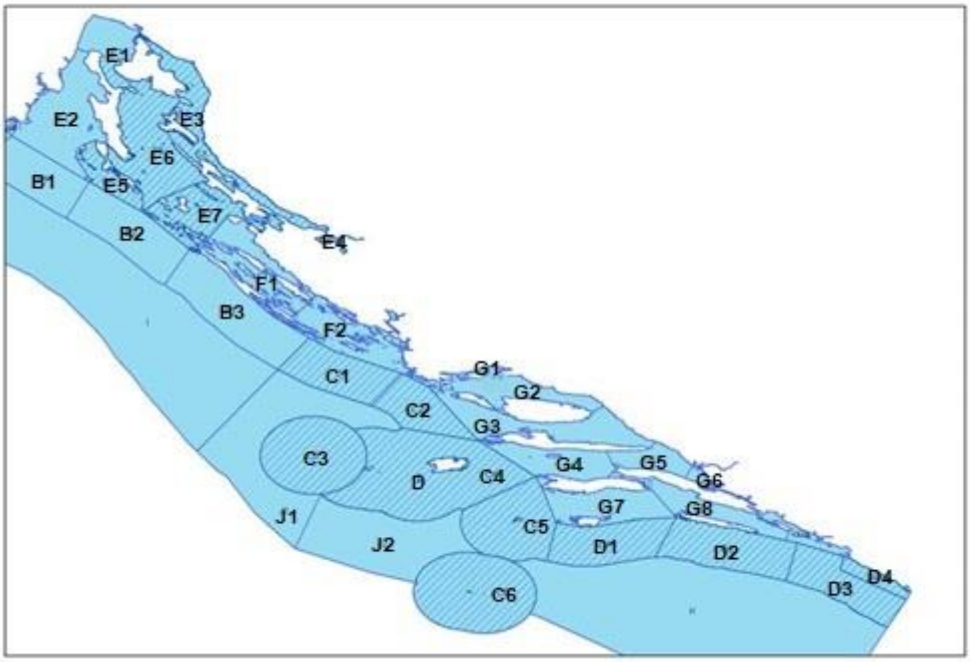


Figure 5.11. Croatian Fishing grounds adopted for the Croatian DCF sampling program by IOF of Split.

The G3 - J2 grounds are partially out from the boundaries of the previously defined "Pomo" area and their intake in *Nephrops* catches can be considered negligible, thus were not taken in account (Figure 5.12).

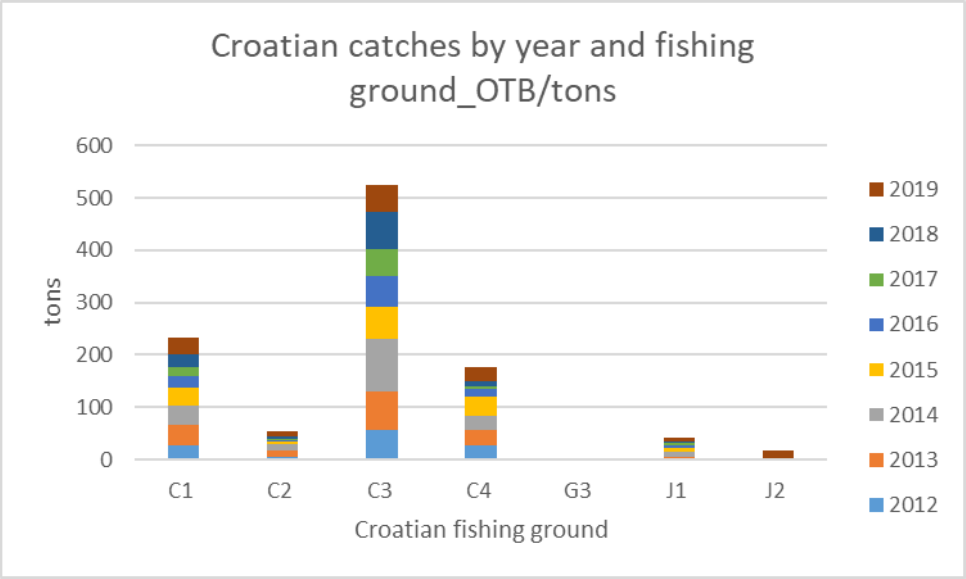


Figure 5.12. Spatial distribution of Croatian OTB landings within the different Croatian fishing grounds from 2012 to 2019 (the data referred to the previous years were not provided)

Figure 5.13 shows the Croatian estimated landings from 2008 to 2019 for the “Pomo” study area obtained for each considered time step.

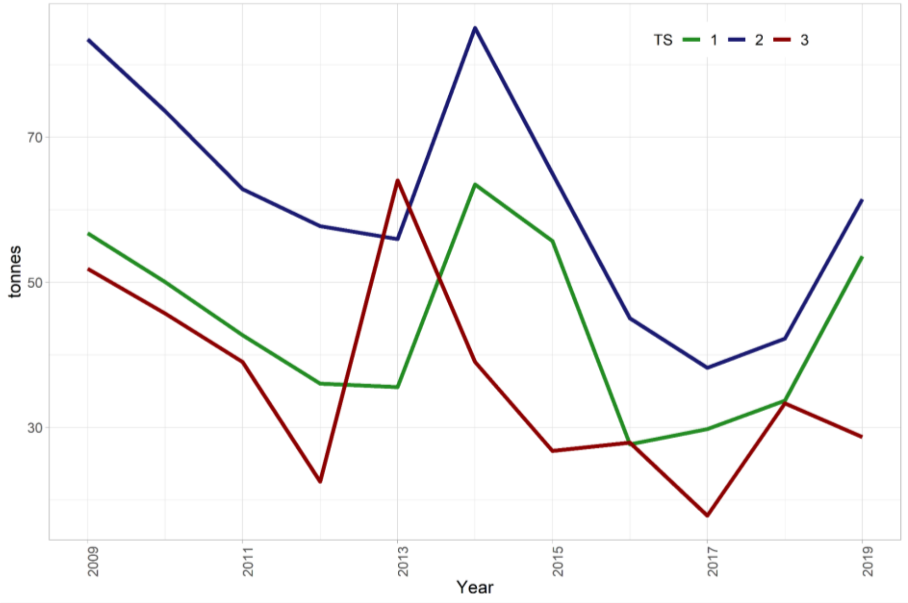


Figure 5.13. Croatian landings by time step (TS1 = Jan_March (green line); TS2 = Apr_Jul (blue line); TS3 = Aug_Dec (red line)) for the “Pomo” area. From 2009 to 2011 the landings were estimated through a ratio by time step calculated on the first 3 available years (2012-2014).

A data frame about *Nephrops* LFDs collected within the Croatian DCF from 2013 to 2019 for fishing ground C (partially matching the previously defined “Pomo” area) was provided by IOF of Split. As done for the Italian time series, the biological information on *Nephrops*’ landings were analysed in order to find consistency with behavioural information on the above described difference in emergency rate between sexes. The analyses on the sex-ratio (Males/(Males + Females)) proportion by year and month were then carried out (Figures 5.14) as well as the aggregated sex proportion by month (Figure 5.15).

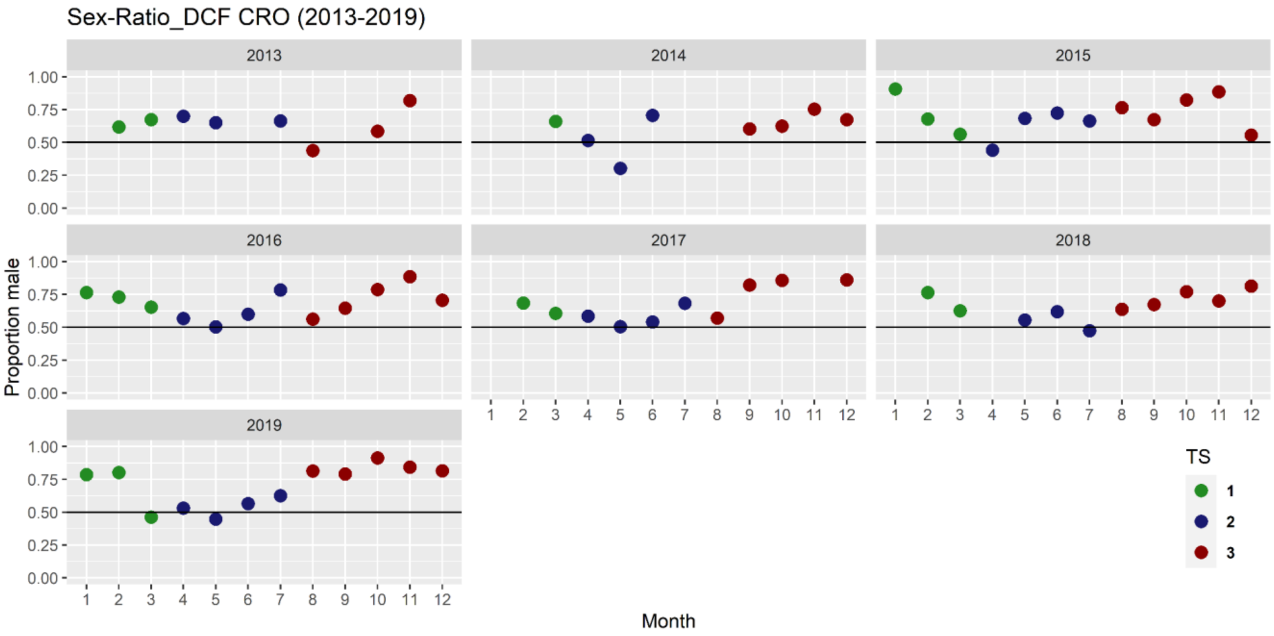


Figure 5.14. Croatian sex-ratio proportion by TS and year (2013 - 2019). TS1 = Jan_March (green dots); TS2 = Apr_Jul (blue dots); TS3 = Aug_Dec (red dots).

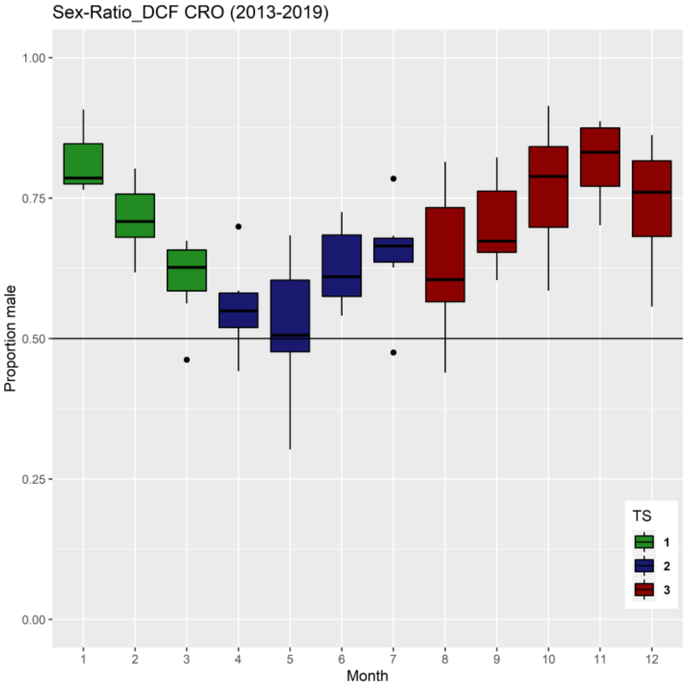


Figure 5.15. Boxplot of Croatian sex-ratio proportion (Males/(Males + Females)) by month with indication of relative time step (TS)

As for the analysis of Italian data, the results show a good accordance between the sampled sex-ratio proportion and the pre-defined TS; there's only an exception: the month of July for which it would have been expected a higher number of captured females. However, the annual sex-ratio proportion for the month of July is not always in favour of the males (e.g. year 2018). The variability between Italian and Croatian biological information is probably due to the different sample size along the different data-sets (no further analyses on variance homogeneity were conducted). Once the sex-ratio proportion by time step for all the available carapace length size classes (mm) was calculated, to overcome the presence of missing classes, a square smooth function weighted on the total n° of sampled individuals by TS was applied to the Croatian LFDs (Figure 5.16). A cross validation was conducted in order to select the more confident number of degrees of freedom for each function.

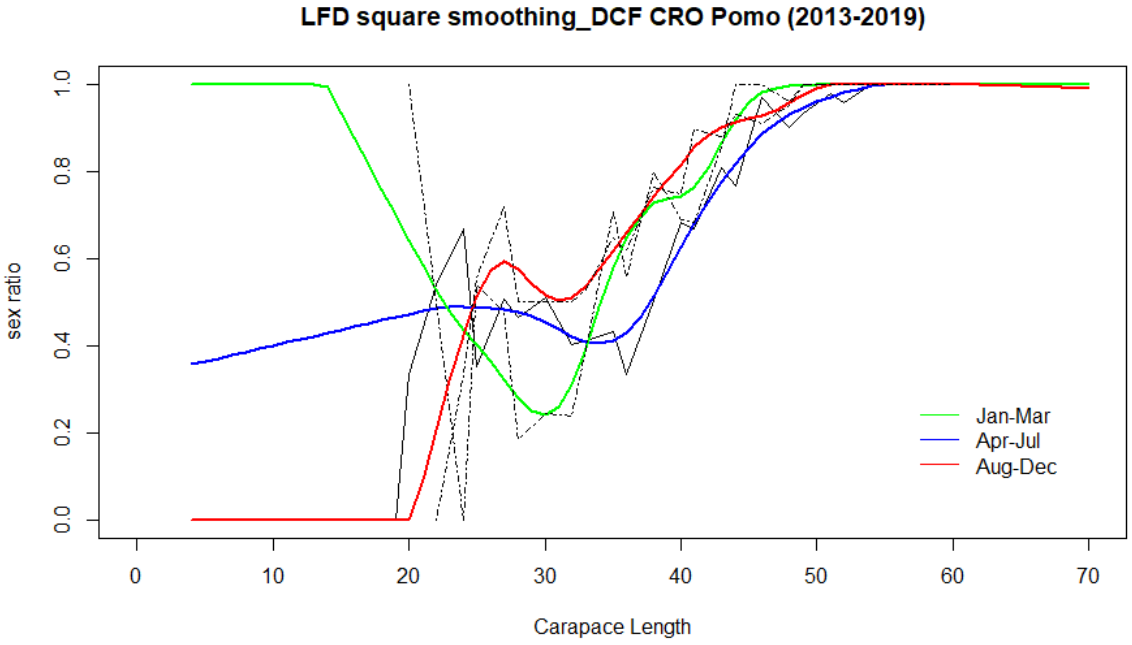


Figure 5.16. Sex ratio (Males/(Males + Females)) smoothing functions by time step from Croatian DCF data (2013 - 2019). The coloured lines represent the smoothing functions for TS1, TS2 and TS3 (green, blue and red lines, respectively), the black lines show the calculated sex-ratio for each previously mentioned time step (dot, dash-dot and continue lines, respectively).

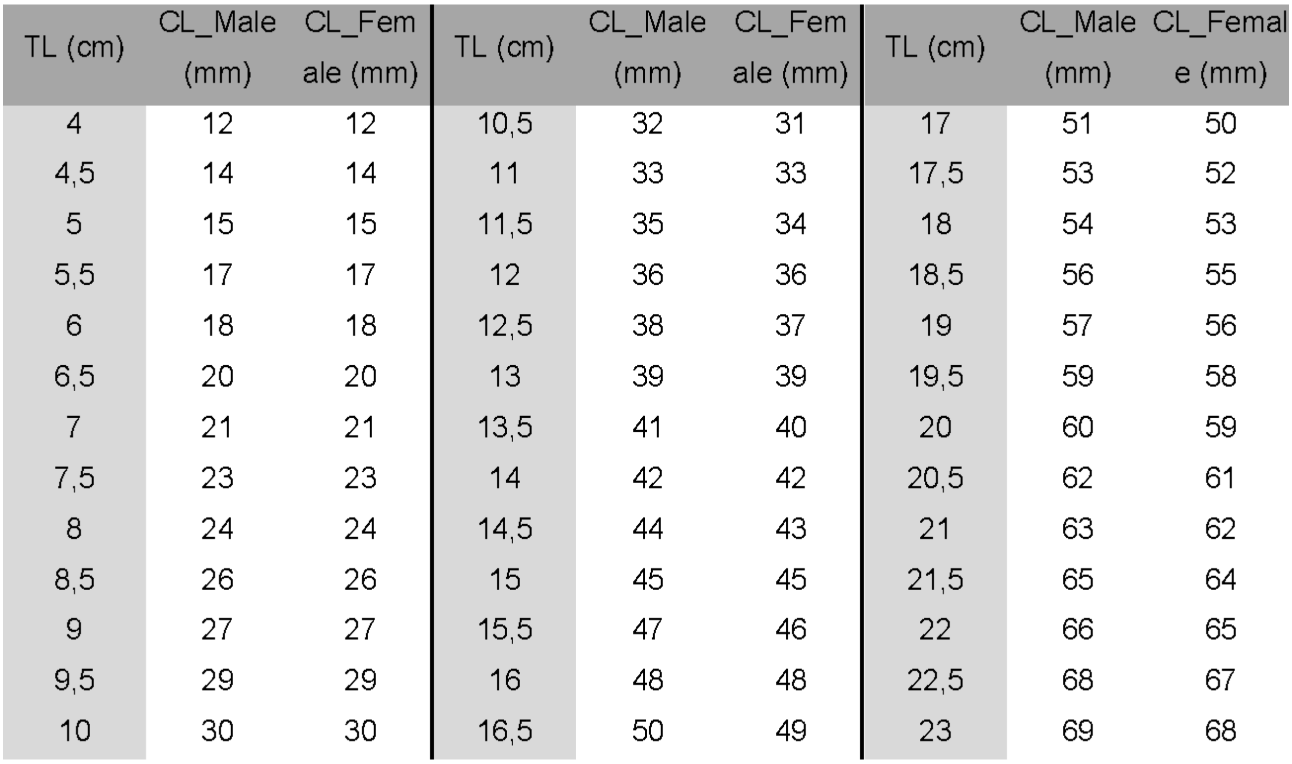
The resulting smooth functions confirm the differences in emergence pattern among TS in accordance with the biological cycle of *Nephrops*; the mating period of this burrowing crustacean species occurs in late spring-summer corresponding to TS2 (from April to July) for which the highest number of females with CL greater than almost 35 mm was indeed observed. The smooth functions estimated from Croatian DCF data are similar to the Italian one as was expected; hence, the Croatian sex-ratio proportion was used to split the respective LFDs within the 3 time steps.

Differently from Italy, the Croatian LFDs were collected by measuring the Total length (TL), hence a length-length relationship was needed to convert the provided LFDs into CL. The length-length relationship by Froglia and Gramitto (1988) is suitable only for separate sexes, therefore the Croatian LFD provided by TL without sex division were first split into males and females through the smoothing function and then converted into CL. The length-length relationship by Froglia and Gramitto (1988) for Pomo/Jabuka Pits was applied as follows:

CL = (10*TL – a)/b

where a = -1.880 and b = 3.355 for males, while a = -2.541 & b = 3.419 for females (Table 5.1).

Table 5.1. Length conversion by sex (numbers are rounded at zero as integer). The length-length relationship used is the one developed by Froglia and Gramitto (1988).



TL (cm)	CL_Male (mm)	CL_Female (mm)	TL (cm)	CL_Male (mm)	CL_Female (mm)	TL (cm)	CL_Male (mm)	CL_Female (mm)
4	12	12	10,5	32	31	17	51	50
4,5	14	14	11	33	33	17,5	53	52
5	15	15	11,5	35	34	18	54	53
5,5	17	17	12	36	36	18,5	56	55
6	18	18	12,5	38	37	19	57	56
6,5	20	20	13	39	39	19,5	59	58
7	21	21	13,5	41	40	20	60	59
7,5	23	23	14	42	42	20,5	62	61
8	24	24	14,5	44	43	21	63	62
8,5	26	26	15	45	45	21,5	65	64
9	27	27	15,5	47	46	22	66	65
9,5	29	29	16	48	48	22,5	68	67
10	30	30	16,5	50	49	23	69	68

The Croatian LFDs previously split into two sexes and then converted into CL (mm) were then aggregated by 2x2 size classes in order to keep consistency with Italian time series (Fig 5.17).

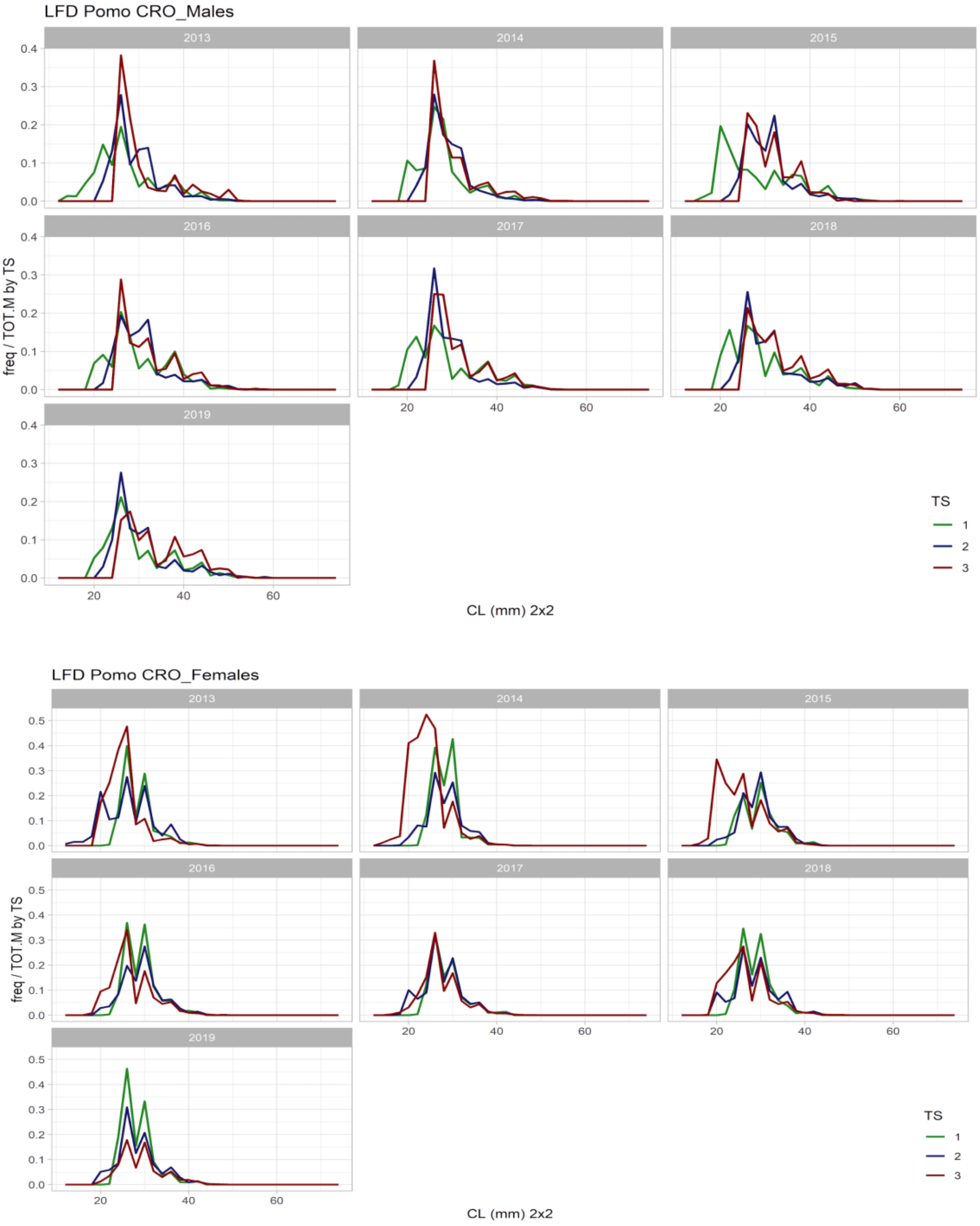


Figure 5.17. Croatian "Pomo" LFD frequencies (2013 - 2019) by sex (upper panels are males, bottom panels are females) and time step (TS1 = Jan_March (green lines); TS2 = Apr_Jul (blue lines); TS3 = Aug_Dec (red lines)) from Croatian DCF data. The CL were aggregated by 2x2 size classes.

For each year the number of trips and observed individuals by quarter and sampled subarea were as well provided by the IOF of Split (Fig 5.18). In accordance with the work previously done on the Italian data, the number of trips (i.e. sample size) instead of the number of individuals was adopted as "process error" (i.e. effective sample size) driving the weight given to the observed data in the subsequent model fitting process (Bull et al., 2012). This dataset was previously filtered by subareas selecting only the areas within the predefined spatial domain named “Pomo” which includes: C1, C2, C3, C4, J1, J2, G3 Croatian subareas.

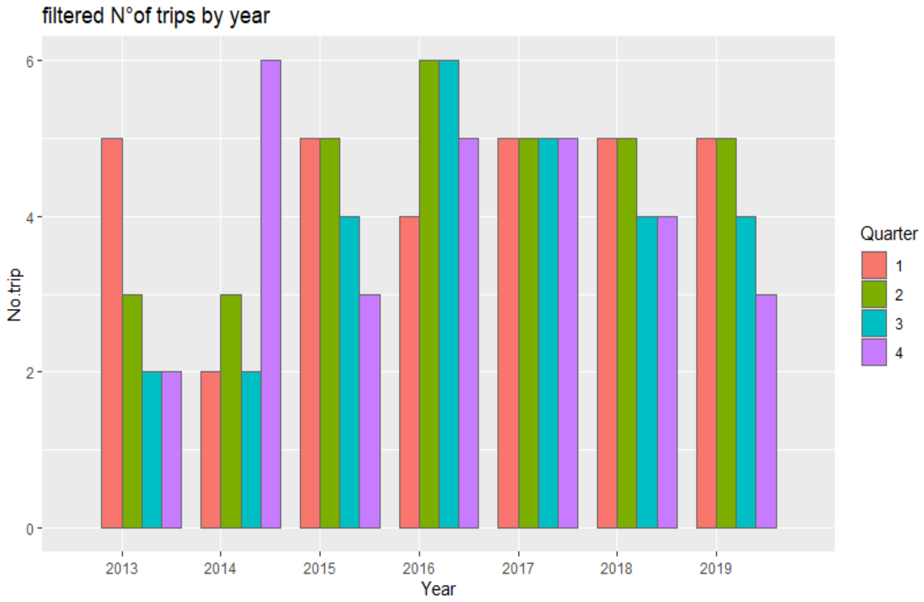


Figure 5.17. Histogram of annual number of trips by quarter carried out by IOF of Split within the Croatian DCF in the “Pomo” area.

Unfortunately, the temporal dimension of quarters was different from the previously settled temporal domain (i.e. Time Steps) (Table 5.2)

Table 5.2. Comparison between the TS division and the quarter division of the solar year.

	JAN	FEB	MAR	APR	MAJ	JUN	JUL	AUG	SEP	OCT	NOV	DEC
TS	1	1	1	2	2	2	2	3	3	3	3	3
Quarter	1	1	1	2	2	2	3	3	3	4	4	4

Thus, in order to arrange the annual number of trips by quarters into the predefined TS, the third quarter has to be proportionally splitted between TS2 and TS3. Assuming that the number of trips for each month in the third quarter (i.e. July, August and September) was balanced, it was divided by 3 for each available year resulting in a ratio of the number of trips by month and year. The number of trips in July was then summed to those in the second quarter corresponding to TS2; while the remaining number of trips of the third quarter (i.e. August and September) was summed to those in the fourth quarter corresponding to TS3. This operation was repeated for each available year (Figure 5.20).

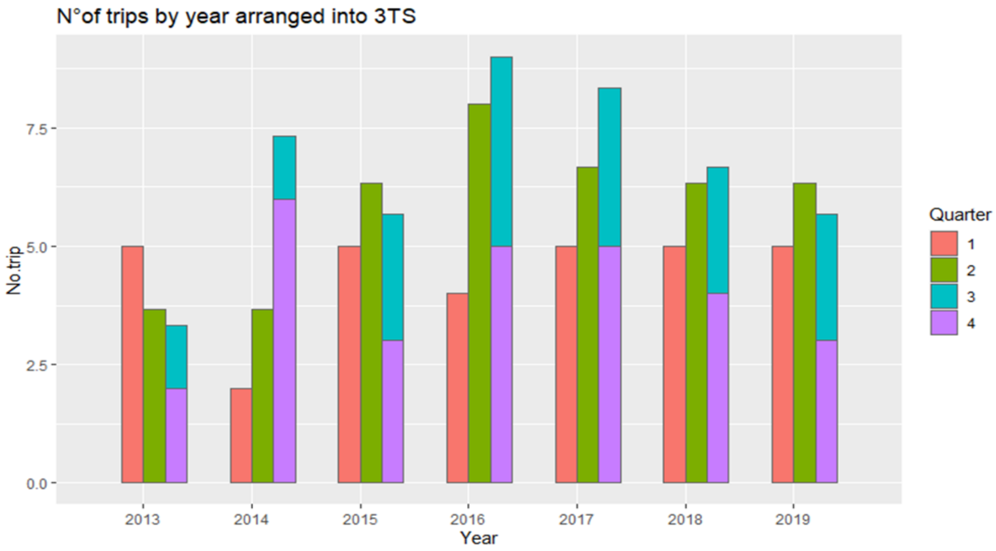


Figure 5.20 Histogram of annual number of trips by quarter (coloured bars) arranged into the predefined 3 TS (1st TS = first column, 2nd TS = second column, 3rd TS = third column)

5.3 Historical reconstruction of Italian (1953-2012) and Croatian (1970-2015) landings data

The historical Italian landings time series was derived from Fortibuoni et al. (2017) (hereafter referred as “Fortibuoni” time series), who provides the Italian annual landings (1953–2012) for the Northern and Central Adriatic Sea originated from official Italian statistics on fishery, reported by the Italian National Institute of Statistics (ISTAT) from 1953 to 2004, and by the Institute for Economic Research in Fishery and Aquaculture (IREPA) from 2005 to 2012. Landings statistics are aggregated at the level of historically most important fish markets of the Italian coastal areas (Italian administrative regions: Friuli Venezia Giulia, Veneto, Emilia Romagna, Marche, Abruzzo and Molise) and do not include discarded, illegal and unreported part of the catches. Landings are reported in terms of annual total weight (kg per year) without information on the fishing gear used. Attempting to follow a method consistent with that developed by Russo et al. (2018) and applied to the most recent Italian landings (2009-2019), a work was carried out to: (i) identify which was the contribution to *Nephrops* production inside the “Pomo” area of each Italian region reported in the “Fortibuoni” time series; (ii) select the regions that contribute most to the *Nephrops* landing from "Pomo" within the “Fortibuoni” time series; (iii) calculate an average ratio starting from the “Pomo” landings estimated for the most recent years (2009-2019, chapter 5.1) and to apply this ratio to the landings for the selected regions in order to split the data for the "Pomo" area only. In accordance with the fishing grounds identified by Russo et al. (2018), the spatial domain of “Pomo Pits” area includes FG.2, FG.3, FG.4, FG.8, FG.9, FG.5 and FG.18 (Figure 5.21).

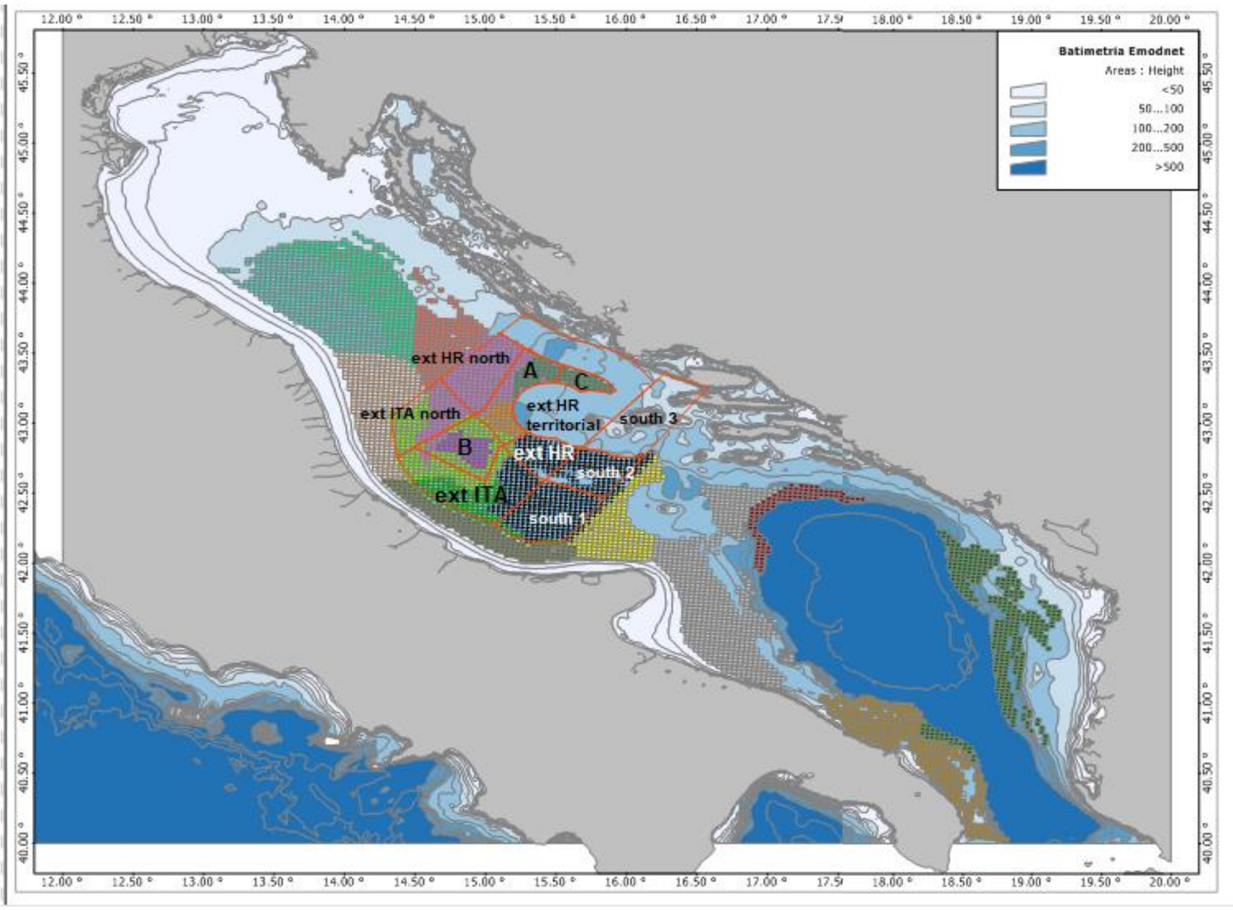
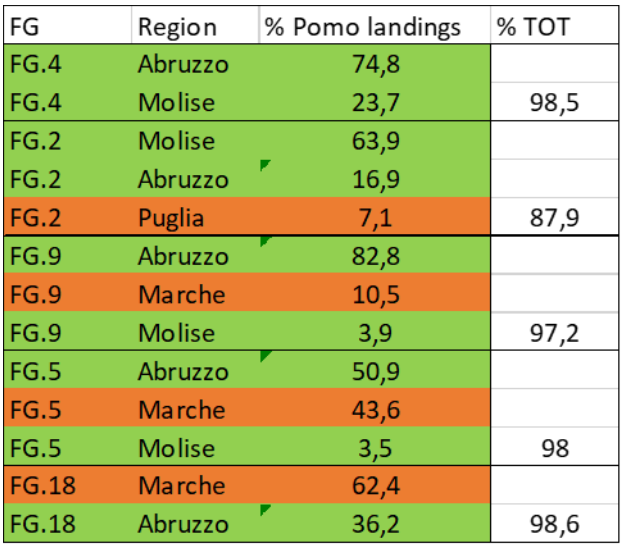


Figure 5.21. The map shows the overlap between the fishing grounds defined by Russo et al. (2018) and the spatial domain (i.e. “Pomo” area, red boundaries define the strata as described in chapter 4.1)

For the four most important fishing grounds as well as those included in the area closed to fishing (5, 9 and 18) the percentage of average production of *Nephrops* from 2009 to 2014 to each of the five most important landing harbours was reported (Russo et al., 2018). With the aim to select the regions for which most of the *Nephrops* landings originate from the previously defined “Pomo” area, the regions to which each of the considered harbours belong were identified and matched with the percentage of landed *Nephrops* of the relative fishing ground (Table 5.3).

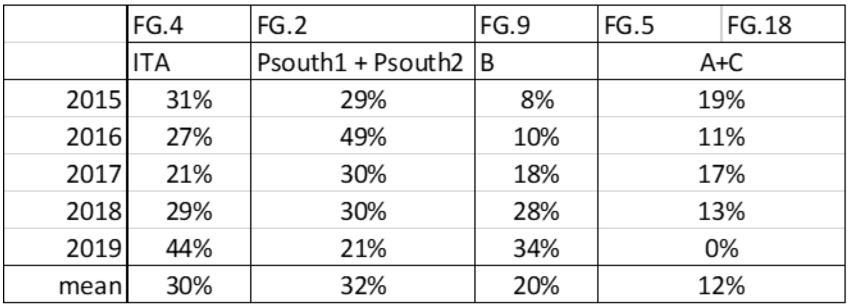
Table 5.3. Percentage of the colours representing the regions with the major (green) and minor (red) contribution to the total landings of *Nephrops* from the “Pomo” area (column “% Pomo landings”). The latest column (“% TOT”) shows the percentage of landed *Nephrops* in respect to the catches inside every fishing ground. Only the regions with the highest percentage of landings were reported; the fishing grounds not reported here have a minor contribution to the annual landings of *Nephrops*.



FG	Region	% Pomo landings	% TOT
FG.4	Abruzzo	74,8	
FG.4	Molise	23,7	98,5
FG.2	Molise	63,9	
FG.2	Abruzzo	16,9	
FG.2	Puglia	7,1	87,9
FG.9	Abruzzo	82,8	
FG.9	Marche	10,5	
FG.9	Molise	3,9	97,2
FG.5	Abruzzo	50,9	
FG.5	Marche	43,6	
FG.5	Molise	3,5	98
FG.18	Marche	62,4	
FG.18	Abruzzo	36,2	98,6

The percentage of landings never drops below 87.9% of total catches estimated for *Nephrops* in the fishing grounds that match the “Pomo” study area; it was therefore assumed that these regions are quite representative of the total amount of *Nephrops* production from the "Pomo" area. The Abruzzo and Molise regions were then selected as the regions that contribute most to the *Nephrops* landing from "Pomo", while Puglia was discarded from the analyses due to its minor contribution in the total catches. The percentage of landings for the Marche region from FG.5 and FG.18 was reasonably high (43,6% and 62,4%, respectively), although in the remaining FGs (i.e. FG.2, 4 and 9) its contribution was scarce or even null. Hence, in order to investigate which was the contribution of each fishing ground of Marche region to the total landings of *Nephrops* from the “Pomo” area, the FGs defined by Russo et al. (2018) were approximately matched with the previously defined strata of the study area (Figure 5.21). Then the percentage of landed quantity from 2015 to 2019 was calculated for each match (Table 5.4)

Table 5.4 Percentage of landings by year and strata. Only the strata actually matching with the previously presented FGs were reported; the strata not reported here have a minor contribution to the annual landings.



	FG.4	FG.2	FG.9	FG.5	FG.18
	ITA	Psouth1 + Psouth2	B	A+C	
2015	31%	29%	8%	19%	
2016	27%	49%	10%	11%	
2017	21%	30%	18%	17%	
2018	29%	30%	28%	13%	
2019	44%	21%	34%	0%	
mean	30%	32%	20%	12%	

The minor contribution of *Nephrops* landings inside “Pomo” was identified within the FG.5 and FG.18 corresponding to the strata “A” plus “C”. The FG.5 and FG.18 were those for which the Marche region presented almost the majority of landings. Furthermore, Russo et al. (2018) provided evidence that almost all the catches from “Pomo” for the Marche region, especially for FGs 5 and 18, were landed in San Benedetto del Tronto harbour (43,6 % and 60,5%, respectively). Therefore, the Marche region was not considered in this study due to the minor contribution of FG.5 and 18 (i.e. 12%) on the total landings for the "Pomo" area, and to the lack of a spatial dimension which allows to select only the port of San Bendetto del Tronto within the “Fortibuoni” time series. Italian landings data from Fortibuoni et al (2017) were previously filtered for the target species and for the regions in which it is landed mostly of the Norway lobster fished in Pomo Pits (i.e. Abruzzo and Molise; Figure 5.22), according to Russo et al. (2018). Landing data for 1973 were not provided.

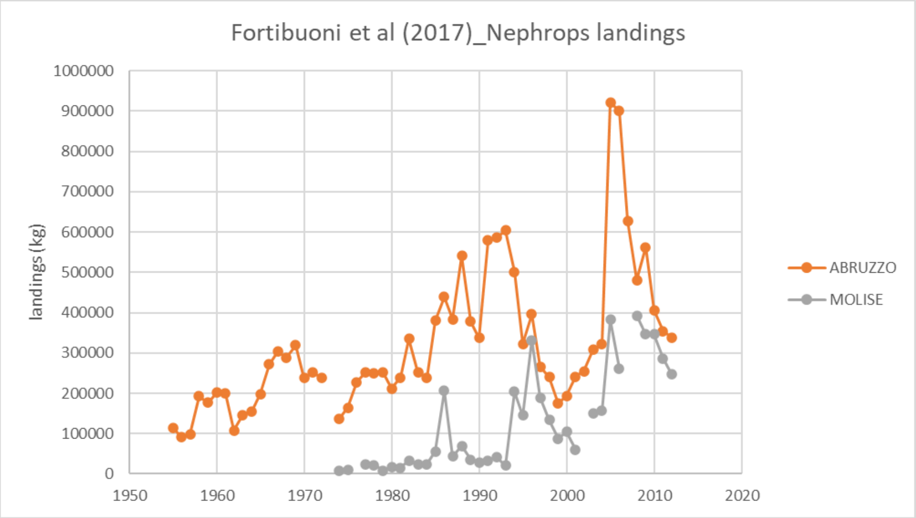


Figure 5.22. Fortibuoni et al., (2017) time series (1953 - 2012) filtered for the Abruzzo (orange) and Molise (grey) regions. Landing for 1973 were not provided.

With the aim to calculate a useful ratio to split by gear the “Fortibuoni” time series and take into account only trawlers that fished in the “Pomo” area, a comparison of the data’s features among all the available italian time series was carried out (Table 5.5).

Table 5.5 List and description of all the available time series. “TBB/DES” corresponds to rapido trawlers. “OTB/DES” refers to the bottom trawl fishery. “TS/ monthly division” represents the provided temporal scale, while the GSA levels (GSA 18 and GSA 17) refers to the spatial scale. The “DCF_ITA (lander)” refers to the method developed by Russo et al. (2018). The “DCF_ITA (regions)” dataset was provided by Mably to CNR-IRBIM.

	available years	*TBB/DES ("rapido")*	*OTB/DES (trawl)*	*TS/monthly divsion*	*GSA 18*	*GSA 17*
DCF_ITA (lander)	2009:2019		X	X	X	X
DCF_ITA (regions)	2004:2018	X	X	X		X
Fortibuoni (regions)	1955:2012 (no 1973)	provided jointly				X

An agreement between fishing gears comprised in "DCF_ITA (regions)" and "Fortibuoni" timeseries was observed, hence a comparison between these was carried out (Figure 5.23). The aim was to investigate if there was an accordance between time series and eventually calculate a ratio to tune the historical landings with the Italian DCF by region.

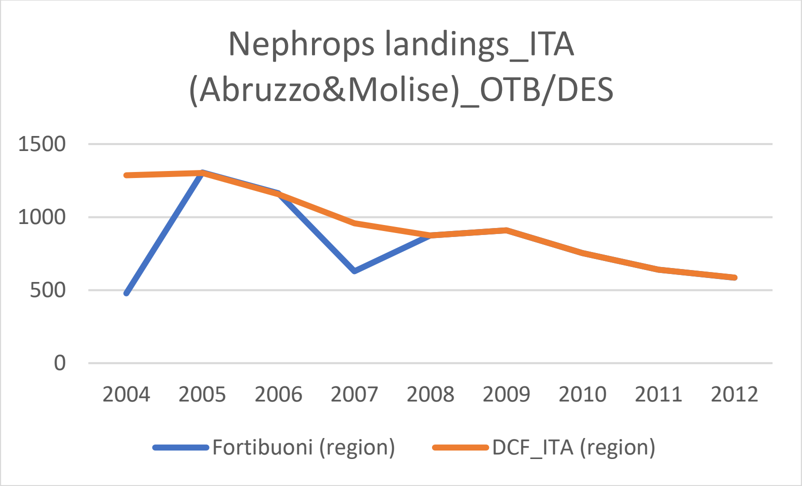


Figure 5.23. Italian *Nephrops* landings (in tonnes, y-axis) for "ITA_DCF (region)" (orange) and "Fortibuoni" time series (blue)

Only 2004 and 2007 landings differ from the "DCF_ITA (region)" time series; however, no proportion was applied to the historical time series. This huge difference for 2004 could be attributed to the different sources of data: "Fortibuoni" data for 2004 was from ISTAT while "DCF_ITA (region)" are based on Mably reported landings. Giving the fact that landings for Abruzzo and Molise from Italian DCF were quite similar to the ones from "Fortibuoni", the "DCF_ITA (region)" time series was used as a proxy of landings only from "Pomo Pits" area. The estimated landings with the method developed by Russo et al. (2018) from 2009 to 2019 were thus compared with the time series from 2004 to 2018 collected within the Italian DCF and previously filtered for the selected regions (Abruzzo and Molise). Landings from Pomo Pits region estimated by means of the method developed by Russo et al. (2018) accounting for Italian OTB fishing gears from both GSA17 and 18. This method is based on the Vessel Monitor System (VMS) which was compulsory for Italian vessels above 15 m of Length Over All (LOA) since 2006 (EC, 2002, 2003); this threshold has been downscaled to 12 meters of LOA from 2012 (EC, 2009). Hence a ratio to be applied to the whole time series was calculated (Table 5.6 and Figure 5.24)

Table 5.6 Landings data by year provided by Mably to CNR-IRBIM ("DCF_ITA (regions)") and landings data estimated with the method developed by Russo et al. (2018) ("DCF_ITA (lander)"). The last column shows the resulting ratio (in yellow the values used to average the final ratio).

Year	DCF_ITA (region)	DCF_ITA (lander)	ratio
2009	909.8418	1346.814	1.480273
2010	753.6857	1212.199	1.608360
2011	640.3628	602.6491	0.941105
2012	585.8362	497.2879	0.848851
2013	456.3818	484.1862	1.060923
2014	342.4722	376.3872	1.099029
2015	319.1026	351.1485	1.100425
2016	267.2618	316.0256	1.182457
2017	215.0837	334.7392	1.556320
2018	311.0832	468.5339	1.506136

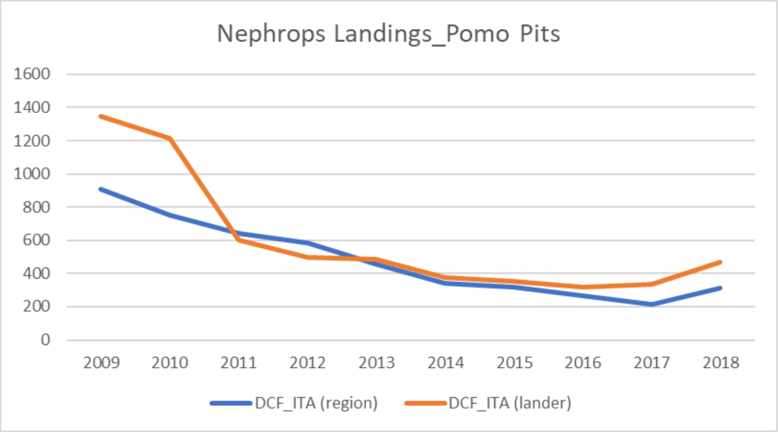


Figure 5.24 Landings data by year provided by Mably to CNR-IRBIM (“DCF_ITA (regions)”) and landings data estimated with the method developed by Russo et al. (2018) (“DCF_ITA (lander)”)

It was then calculated an average ratio on the years from 2012 to 2018 accounting for the best VMS coverage (i.e. also considering vessels above 12 m of LOA; EC, 2009). The ratio (=1.193449242) was thus applied to the “Fortibuoni” historical time series to split landings and obtain those attributable to the “Pomo Pits” area (Figure 5.25).

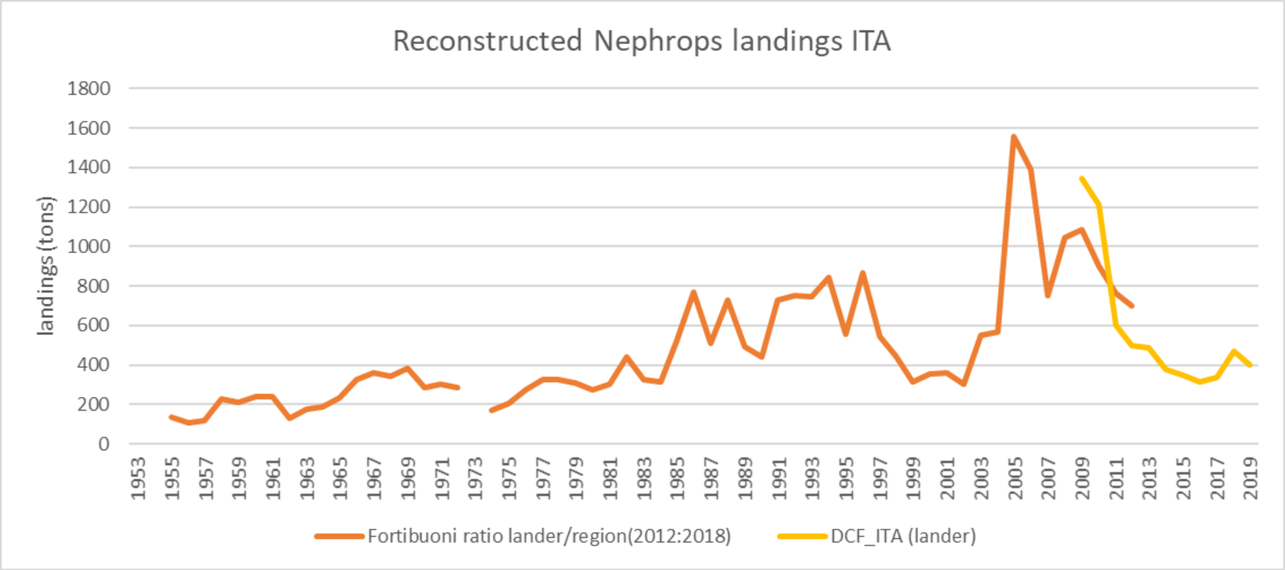


Figure 5.25. Italian reconstructed historical *Nephrops* landings for the “Pomo” area.

Croatian landings data from the RECFISH research program (database of the RECFISH project - EASME/EMFF/2016/032 Specific contract num. 1) were provided for the entire Geographical Sub-Area 17 (GSA 17) from 1970 to 2015. Previous analysis on Croatian DCF (chapter 5.2) proved evidence that FPO vessels (i.e. creel/pots fishery) contribution is negligible, hence only OTB vessels (i.e. trawl fishery) were selected. The filtered historical time series (“RECFISH”) was then compared with the available trawl landings from the Croatian DCF ("DCF_HRV") considering the entire spatial domain (Figure 5.26); this comparison could be useful to highlight whether it was necessary to apply a ratio to tune the historical time series with the most recent one.

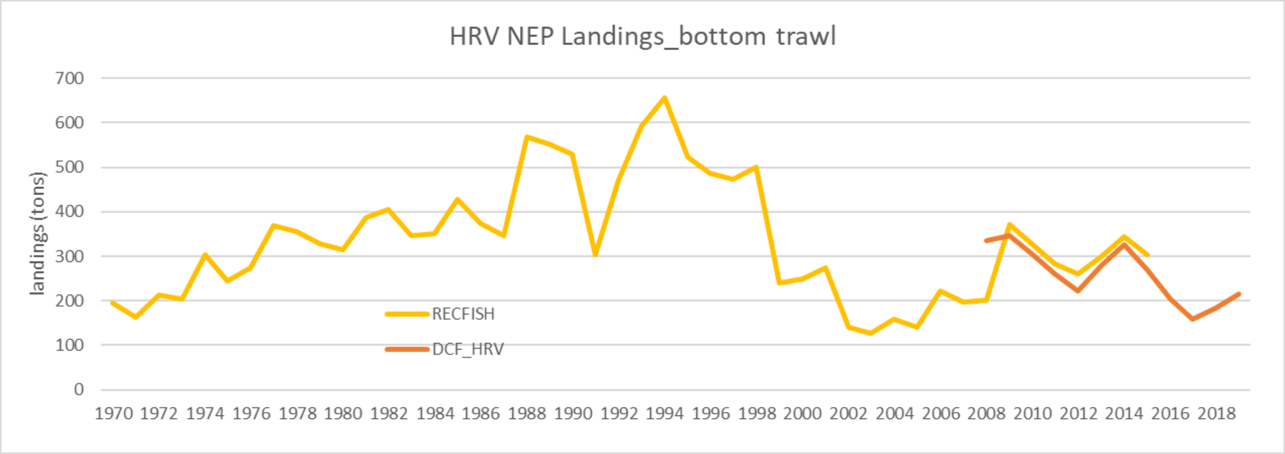


Figure 5.26. Landings by year from RECFISH program (yellow) and Croatian DCF (red)

The biggest difference was found in 2008 (about 40%); with the exception of 2008, the average difference between time series is about 9,6% (with the second highest discrepancy found in 2012 -> 16,7%). Hence no proportions were applied to the historical dataset assuming a great level of concordance between the two time series. In order to split landings data from the RECFISH program only for "Pomo" area, a proportion between landings collected within the Croatian DCF for all the Croatian fishing grounds (=Croatian total landings for GSA17) and the landings filtered only for the Croatian fishing grounds corresponding to the "Pomo" area was calculated for each common year (i.e. 2012:2019); a ratio to be applied to the whole historical time series was calculated as average on the first three common years (Table 5.7)

Table 5.7 Landings by year for the official dataset filtered for the croatian fishing grounds matching the "Pomo" area ("DCF_CRO (Pomo)") and the data including all the fishing grounds ("DCF_CRO (all)"). The last column shows the resulted ratio (in yellow the values used to average the final ratio)

Year	DCF_CRO (Pomo)	DCF_CRO (all)	ratio
2012	116.3197	222.8231	0.522027

2013	155.5541	278.1666	0.559212
2014	187.5582	325.2178	0.576716
2015	147.4361	268.6156	0.548874
2016	100.6316	202.7804	0.496259
2017	85.87466	158.8447	0.54062
2018	109.2916	182.8256	0.597792
2019	143.7046	214.2459	0.670746

A coefficient of 0.552651 was then applied to the historical RECFISH time series splitting the historical GSA17 landings for the Croatian fishing grounds corresponding to the “Pomo” area (Figure 5.27).

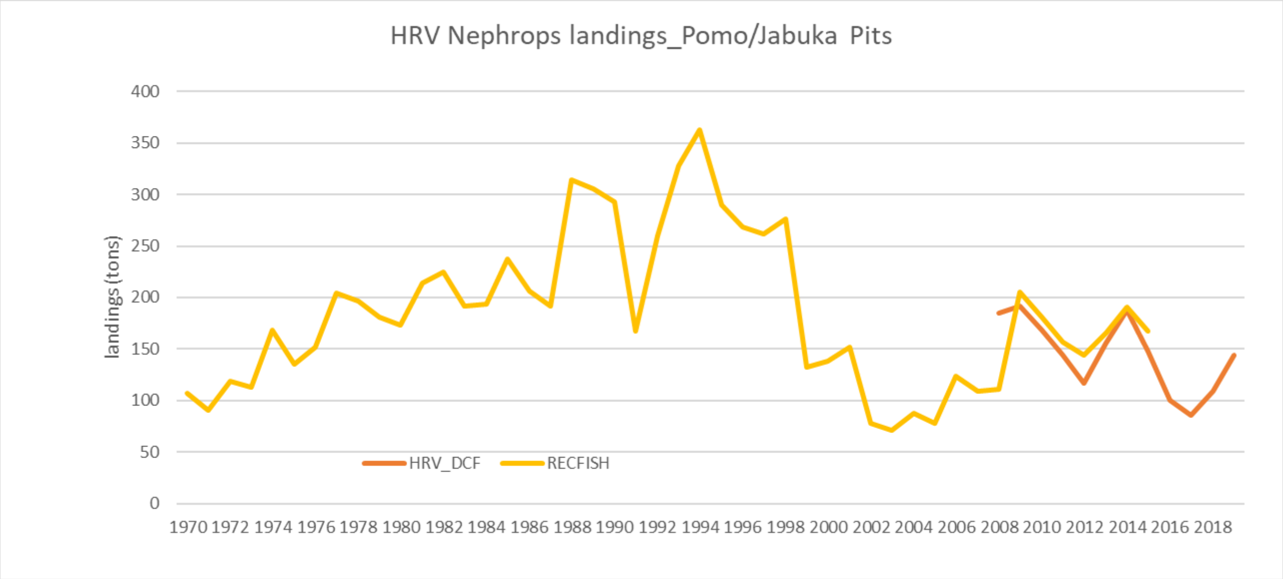


Figure 5.27. Croatian reconstructed historical *Nephrops* landings for “Pomo” area

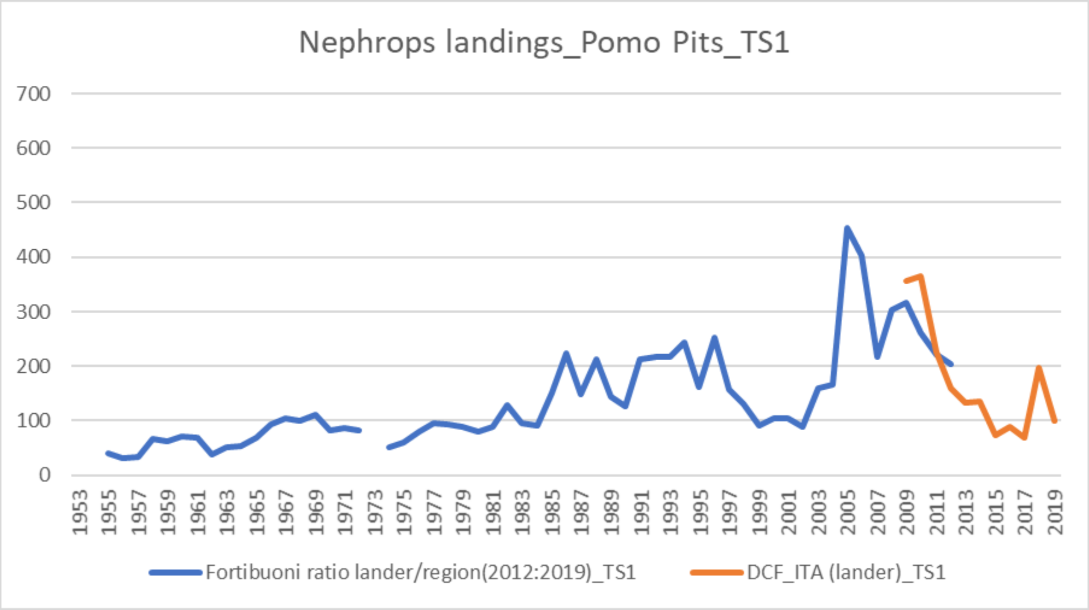
After a reliable reconstruction of Italian *Nephrops* landings related to the trawl vessels fishing within the “Pomo” area was carried out, the time series was then split into three Time Steps (TS) following the biological knowledge of annual emergence pattern of *Nephrops* (see above). Due to the absence of a monthly temporal dimension for the “Fortibuoni” time series, the dataset developed by means of the method of Russo et al. (2018) was used to calculate a proportion for the three time steps. To be consistent with the previous calculations, to calculate an average proportion useful to split landings for each time step, it was chosen to use the years of the Italian DCF from 2012 to 2019 (Table 5.8).

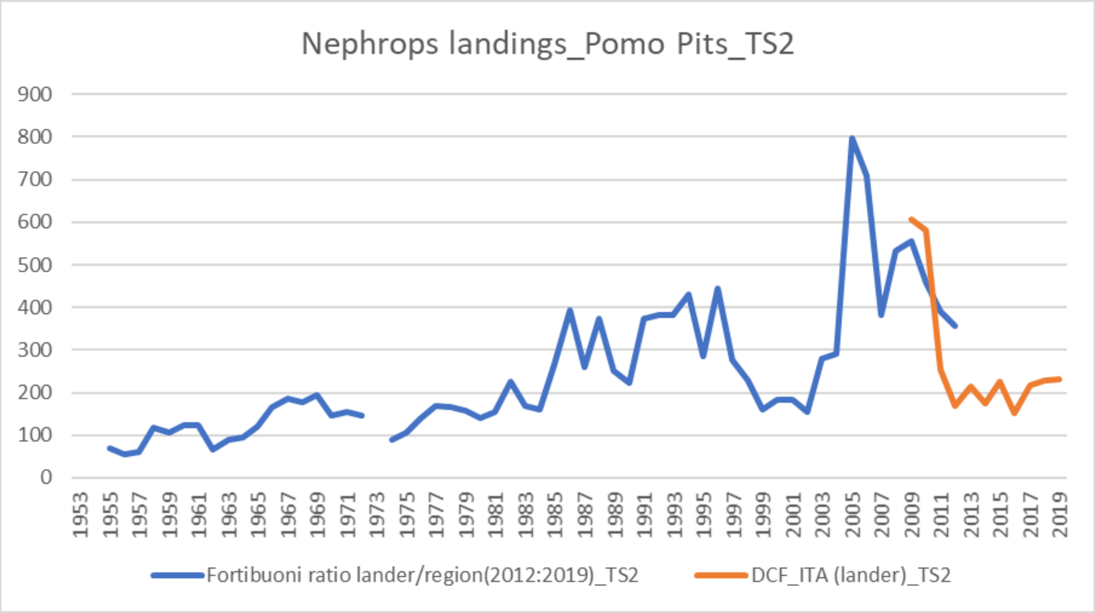
Table 5.8. Landings by year estimated by means of the method developed by Russo et al. (2018) (“Pomo Landings ITA_DCF”). The last three columns show all the available proportions in percentage (%) for each TS (in yellow the values used to average the final ratios).

Year	Pomo Landings ITA_DCF	TS1 (%)	TS2 (%)	TS3 (%)
2009	1346.81449	26.37161	45.0355	28.59288
2010	1212.1987	30.21016	48.02773	21.76211
2011	602.649108	37.5748	42.14324	20.28196
2012	497.287938	32.16429	34.02578	33.80994
2013	484.18616	27.60522	44.22963	28.16515
2014	376.387188	36.06606	46.11796	17.81598
2015	351.14852	20.59295	64.47122	14.93582
2016	316.02557	28.12744	48.47299	23.39957
2017	334.739205	20.86897	64.57143	14.55959
2018	468.533877	42.17686	48.8162	9.006941
2019	399.466463	24.72131	58.19175	17.08694

	mean (2012:2019)	**29.04038**	**51.11212**	**19.84749**

The mean proportion for each TS was then applied to the reconstructed historical time series (Figure 5.28).





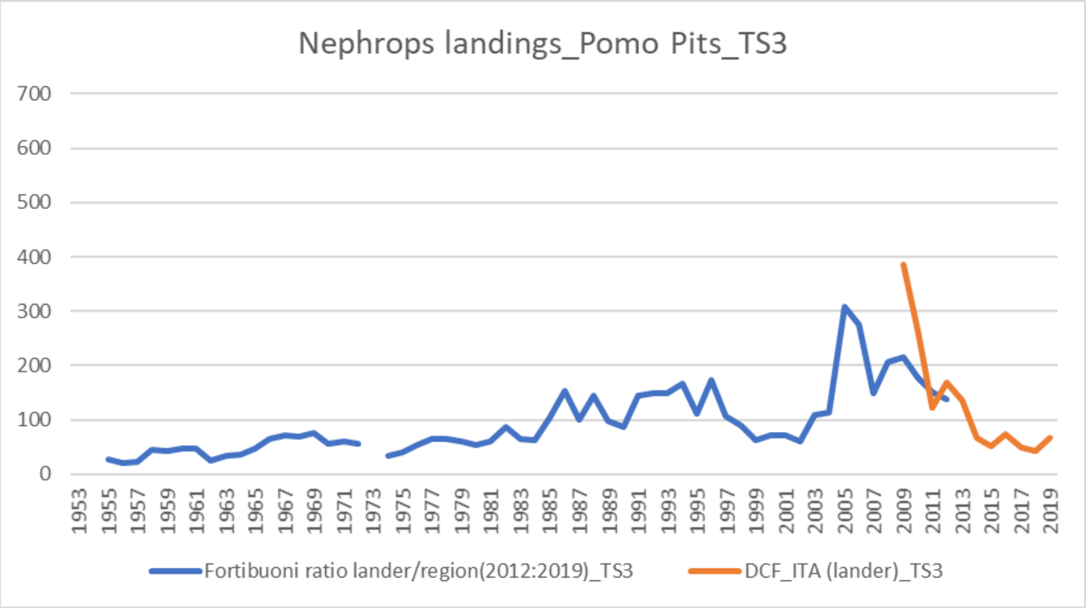


Figure 5.28 Italian reconstructed historical *Nephrops* landings for each TS comparing the time series developed with the method of Russo et al. (2018) (red line) with the reconstructed one (blue line)

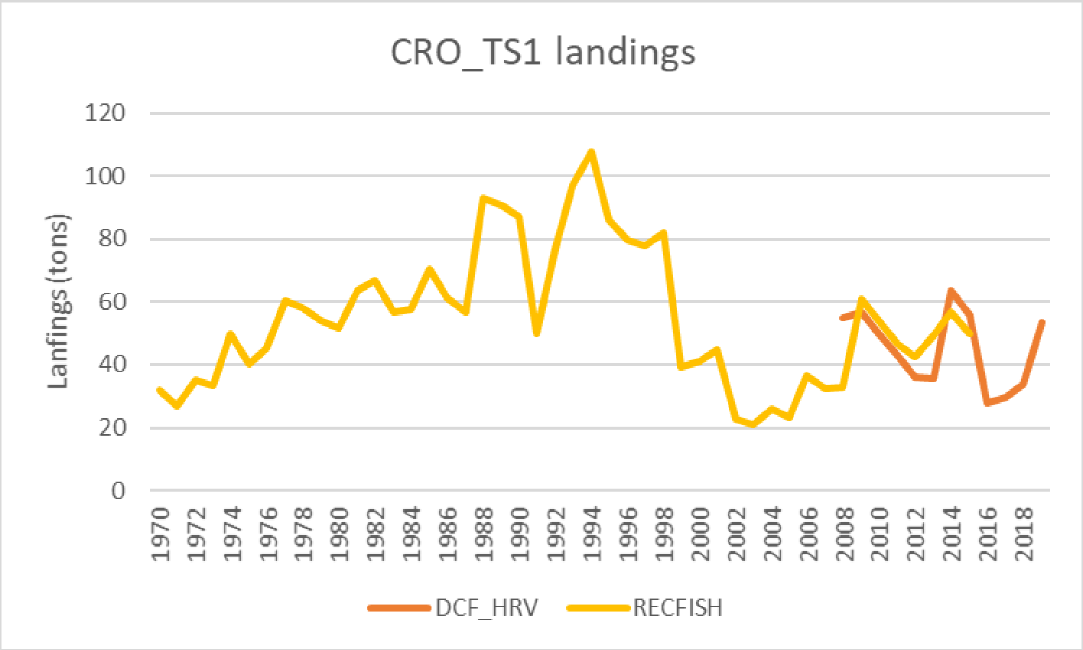
As did for the Italian one, the reconstructed historical time series by RECFISH was then split into the three time steps previously defined. Due to the absence of a monthly temporal dimension for the provided landings, the "CRO_DCF" landings proportion was calculated for the three time steps. To be consistent with the method previously applied, it was chosen to use only the first three years of the Croatian DCF time series to calculate an average proportion useful to split landings by each time step (Table 5.9).

Table 5.9. Landings by year for the official dataset filtered for the croatian fishing grounds matching the "Pomo" area ("DCF_CRO (Pomo)"). The last three columns show all the available proportions in percentage (%) for each TS (in yellow the values used to average the final ratios).

Year	Pomo Landings HRV_DCF	TS1 (%)	TS2 (%)	TS3 (%)
2012	116,3197	30,9933	49,63062	19,37609
2013	155,5541	22,86382	35,97087	41,16531
2014	187,5582	33,85185	45,33895	20,8092

2015	147,4361	37,77564	44,07032	18,15403
2016	100,6316	27,50954	44,75638	27,73408
2017	85,87466	34,68809	44,51605	20,79586
2018	109,2916	30,83799	38,66397	30,49804
2019	143,7046	37,2889	42,75572	19,95538
	mean (2012:2014) ->	**29,23632**	**43,64681**	**27,11686**

The mean proportion for each TS was then applied to the reconstructed historical time series (Figure 5.29).



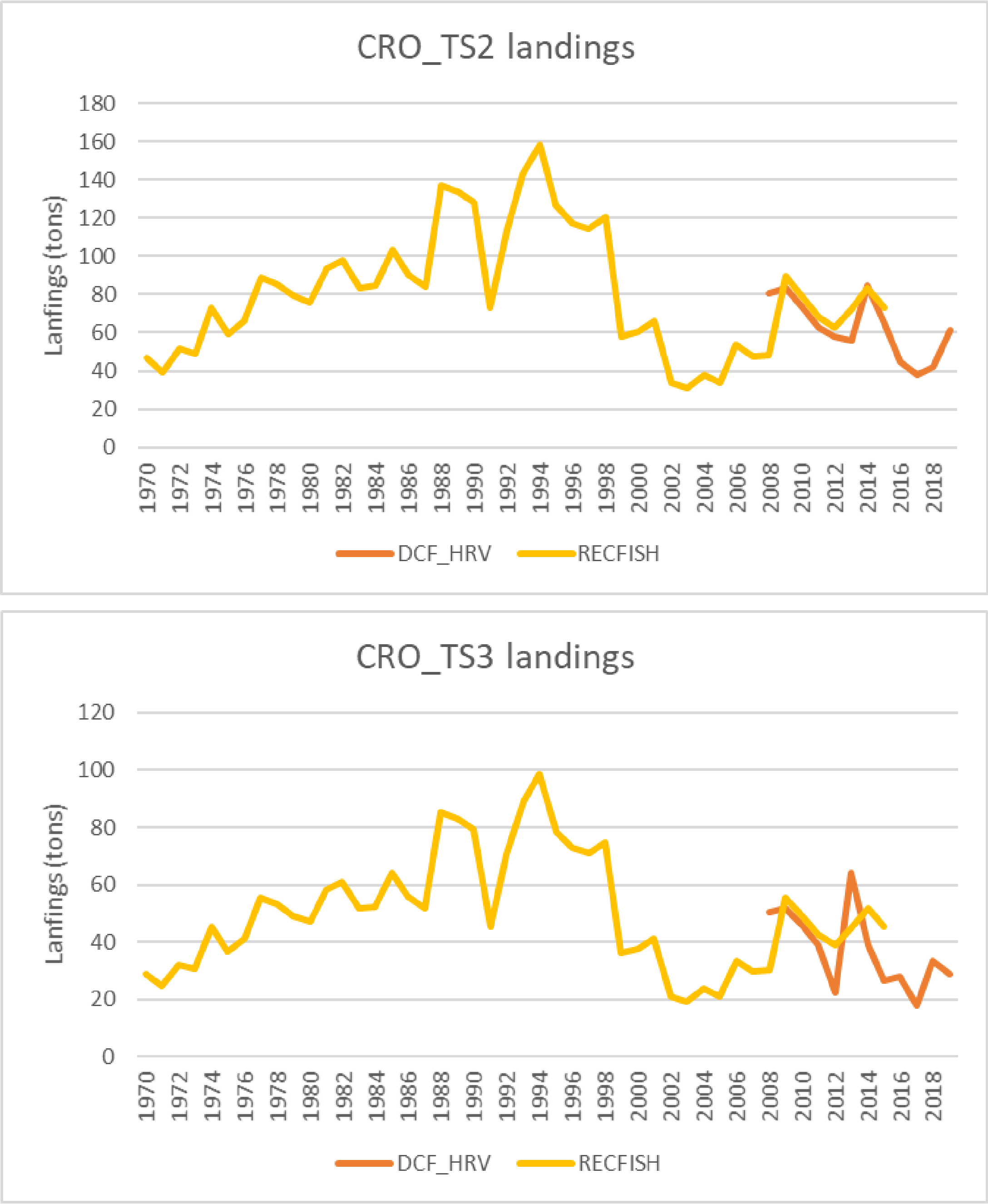


Figure 5.29 Croatian reconstructed historical *Nephrops* landings for each TS comparing the official time series (red line) with the reconstructed one (yellow line)

5.4 Assessment of *N. norvegicus* population in Pomo/Jabuka Pits: A length-based model *via* CASAL

The choice of a methodology to assess the *N. norvegicus* population inhabiting the “Pomo” study area was based on careful consideration of a number of issues. The different sources of data and their shortcomings discussed above were considered together. As previously mentioned, the assessment of *Nephrops* populations is fraught with difficulties: 1) their burrowing behaviour and emergence patterns (individuals only leave their burrows to feed and mate and this happens in different proportions according to sex and season) heavily influence their availability to fishing gear, 2) there is a marked sexual dimorphism in growth parameters, 3) they are characterised by discontinuous growth which occurs only during moulting, making accurate age determination impossible, and 4) in the Adriatic Sea, they are the target of two fleets, the Italian and Croatian trawling fleets. For these reasons, the classical stock assessment methods based on the use of age classes are not considered appropriate, highlighting the need for explicitly length-based methods which consider length classes directly as well as treating sexes separately and yielding fleet-based results. The work carried out in New Zealand on *Metanephrops challengeri* is an example of this (Tuck, 2017). *Metanephrops challengeri* is assessed using a Bayesian length-based approach by means of CASAL (C++ algorithmic stock assessment laboratory; Bull et al. 2012). CASAL is a very flexible platform which allows the specification of complex models, both single and multi-species, taking into account numerous variables and using information by length directly without slicing it into ages. It can generate point estimates of the main parameters of interest as well as likelihood profiles and Bayesian posterior distributions, and can project stock status into the future as well as calculate outputs of interest to management e.g. Fmax, F0.1, MSY (Bull et al., 2012).

The main difference between an age-based and a size-based model lies in the way growth is specified. In a size-based model growth is the process by which fish move between subsequent size classes. This requires good estimates of growth as they will influence (and possibly confound) estimates of fishing mortality (Dobby and Hillary, 2008). Growth and the uncertainty about it are one of the major impediments to a good assessment of *Nephrops* stocks. In CASAL growth can be specified in three different ways: (i) the Francis parameterisation (Francis, 1988) which makes use of growth increments from the Von Bertalanffy growth function, (ii) an alternative Francis parameterisation with exponential decay and (iii) a fixed user-defined transition matrix. A number of growth functions have been estimated for Adriatic *Nephrops* within the "Pomo" spatial domain throughout the years and were used as fixed input parameters within the CASAL models described below. In this assessment, the "basic" fish growth increment model was selected as the Francis (Francis 1988) parameterisation of the growth increment Von Bertalanffy curve. The Francis (1988) equation is

$$\Delta L = (\frac{\beta g_\alpha - \alpha g_\beta}{g_\alpha - g_\beta} - L_1)(1 - (1 + \frac{g_\alpha - g_\beta}{\alpha - \beta})^{\Delta t})$$

where ΔL is the expected change in length (mm) over the change in time, Δt (y) and L_1 is the initial length (mm) of an individual at tagging. The parameters g_a and g_b are the mean annual growth rates (mm/year) of fish at user-selected total lengths (mm) of a and b. If lengths a and b are assigned values within the range of lengths of tagged individuals, then g_a and g_b can be considered descriptive of the individual growth rates encompassed by the tagging data (Francis 1988).

L_∞ (mm) can be estimated from g_a and g_b using the equation:

$$L_\infty = \frac{\beta g_\alpha - \alpha g_\beta}{g_\alpha - g_\beta}$$

Similarly, k (y^{-1}) can be calculated from the model parameters using the equation:

$$k = -\ln\left(1 + \frac{g_\alpha - g_\beta}{\alpha - \beta}\right)$$

Hence, the growth rate by Francis parametrization for Pomo subpopulation was estimated through the Von Bertalanffy growth curve settled with the parameters published in Froglia and Gramitto (1988) by sex (Figure 5.30, Table 5.10).

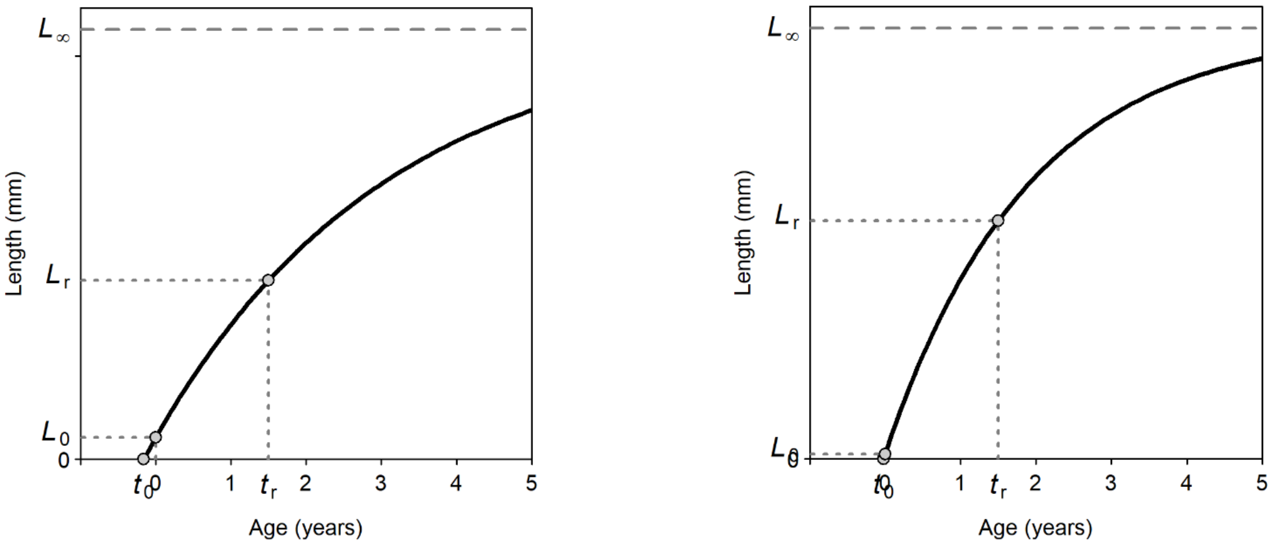


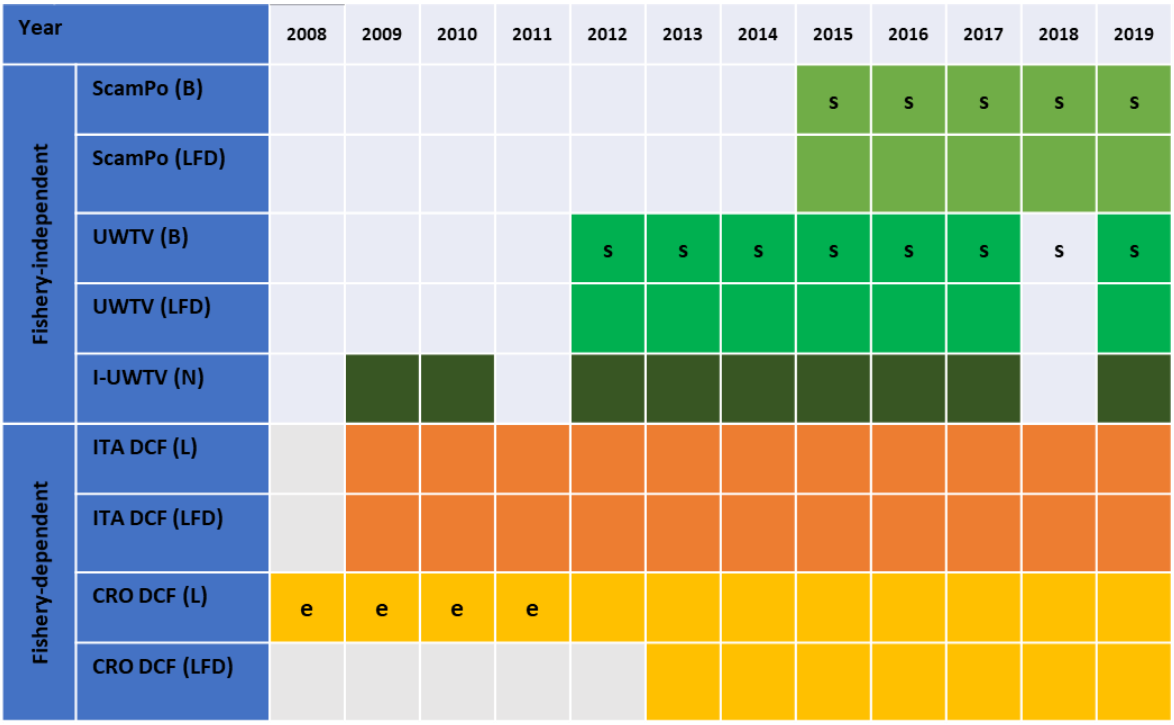
Figure 5.30. Von Bertalanffy growth parameters from Froglia and Gramitto (1988) by sex. For males (left panel) L_∞ (mm) = 53.3062, K = 0.324, and t_0 = 0.1592, and for females (right panel) L_∞ (mm) = 38.4757, K = 0.528, and t_0 = 0.0225. The fixed "Lr" correspond to the L_{50} taken from Angelini et al. (2020) with tr=1.5, thus for males L_r = 22.16686, while for females L_r = 21.2544.

Nephrops are distributed across a range of areas within the Adriatic, with life history characteristics (growth, size at maturity, population density) being markedly different between the Pomo/Jabuka Pit area ("Pomo", slower growth and smaller size at maturity) than elsewhere. The assessment was conducted only for the “Pomo” study area dealing with such a difference in the population biological features.

The model structures applied in the assessment reflecting the seasonal patterns in *Nephrops* sex ratio, related to moulting and reproductive behaviour. Sex was included in the model partition to allow for different availability of the two sexes. Catches and surveys were divided into three time steps reflecting periods of the year when both sexes are relatively equally available to the fishery (April to July, TS2), and when mature females are far less available than males (August to December, TS3; and January to March, TS1). Adoption of these time steps means that the model year runs from January to December according to the solar year.

The Pomo Pit *Nephrops* stock was modelled from 1974 to 2019 (model year). The Pomo Pit stock extends from international waters into the Croatian territorial sea. Two distinct fisheries operate in these areas and, given that *Nephrops* do not migrate, it was decided to consider the two areas as separate stocks within a single model (a two stock model). In this way it will be possible to discriminate possible differences in the parameters of the population (e.g. recruitment) and in the characteristics of the fisheries (i.e. different catch histories) operating in the two areas. Data were therefore collated by year, time step and area (stock) accordingly to the data treatment previously done (see chapters 5.1–5.3). The latest data (2008:2019) available for the Pomo Pit model are listed in Table 5.10, while the historical landings (1974:2007) were entered in model after the data treatment (see chapter 5.3).

Table 5.10. Latest available data from 2008 to 2019. Data were grouped as fishery-independent (i.e. survey data, highlighted with green palette) and fishery-dependent (i.e. commercial data, highlighted with red and yellow colours). The previously standardized trawl data (see chapter 4.2; indicated with letter “s”) referred to the biomass indices (kg/km^2; named “B”) for both the available surveys (i.e. UWTV and ScamPo); the associated length frequency distribution (not standardized; named “LFD”) were fitted as proportions; data from reviewed counts of *Nephrops* burrows (see chaper 3.1; named “I-UWTV”) were entered as absolute number of individuals (N). For Italian commercial data (named “ITA”, in red) landings (L) were entered in tonnes and associated length frequency distributions (LFD) as proportions (see chapter 5.1); for the Croatian ones (named “CRO”, in yellow) the landings (L) from 2008 to 2011 were estimated on the basis of a ratio calculated on the previous years (indicated with the letter “e”), while for the rest of the available years landings as well as LFDs were entered in the model following the data treatment presented above (see chapter 5.2).



Year		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Fishery-independent	ScamPo (B)								s	s	s	s	s
	ScamPo (LFD)								🟩	🟩	🟩	🟩	🟩
	UWTV (B)					s	s	s	s	s	s	s	s
	UWTV (LFD)					🟩	🟩	🟩	🟩	🟩	🟩		🟩
	I-UWTV (N)		🟩	🟩		🟩	🟩	🟩	🟩	🟩	🟩		🟩
Fishery-dependent	ITA DCF (L)		🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
	ITA DCF (LFD)		🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
	CRO DCF (L)	e	e	e	e	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
	CRO DCF (LFD)						🟨	🟨	🟨	🟨	🟨	🟨	🟨

Italian landings data from 2009 to 2019 were firstly estimated based on analysis of VMS data examining the distribution of fishing effort and landings through the LANDER method (Russo et al., 2018) aimed to estimate the real amount of *Nephrops* Italian catches from the "Pomo" area. The landings and commercial sampling data (Length Frequency Distributions) were thus raised to the official landings data and allocated to time step (TS); besides on the LFDs a square smoothing function was applied to split each size class into the two sexes. Italian historical data from Fortibuoni et al (2017) were previously filtered for the target species and for the harbours in which *Nephrops* is landed mostly; landings from 1974 onwards were then tuned on the latest data estimated with LANDER method regarding "Pomo" production and split into the 3 TS. Croatian landings data from 2012 to 2019 was provided by time step and fishing ground (FG) hence was possible to filter data for the "Pomo" domain (spatially correspond to the Croatian FGs: C1, C2, C3, C4, J1, J2); data from 2008 to 2011 were then estimated through a ratio by time step calculated on the first 3 available years (2012:2014). The Croatian LFDs was firstly raised to the official landings data and allocated to time step; then a length-length relationship (Froglia and Gramitto, 1988) was adopted in order to convert LFDs measured in Total length (TL) into the Carapace one (CL) after applying a square smoothing function to split each size classes into the two sexes. Croatian historical landings data from RECFISH program was firstly filtered by bottom trawl gears and then a ratio to split Historical Croatian data for the Croatian fishing grounds localised into "Pomo" area by TS was applied. Discard and data coming from creel/pots were considered as negligible hence they were not entered in the assessment. Both landings (in tonnes) and LFDs (expressed as a proportion calculated on the total number of observed individuals) with a frequency of occurrence by 2CL (mm) were entered in the model by time step and area or stock (i.e. ITA and CRO); the coefficient of variation (cv) expressed as the n° of trips occurred to collect the samples as well as an additional process error for the whole series applied were fitted to the model. Fishery-independent trawl data from ScamPo (2015:2019) and UWTV (2012:2019) surveys were entered as biomass indices (km/kg^2) after a standardisation process (see chapter 4.2); please note that the length compositions collected during scientific surveys were not standardised. Fishery-independent data from video analysis within the UWTV survey (2009:2019) were entered as absolute number (with associated cv) biased with the latest burrow occupation assumption (Aguzzi et al., 2021, chapter 3.2) after the revision of the historical time series (see chapter 3.1).

Mortality includes natural and fishing mortality (i.e. the processes by which fish are removed from the partition). CASAL combines the two processes when they occur in the same time step. Each time step can include a proportion of the year's natural mortality and/or one or more fisheries (i.e. ITA & CRO). A fishery is thus defined as fishing mortality in a specified area and time step. Natural mortality and fishing mortality occurring in the same area and time step was sequenced as instantaneous mortality applying: first (i) half the natural mortality, then (ii) the mortalities from all the fisheries instantaneously, and finally (iii) the remaining half of the natural mortality. Natural mortality was thus fixed at 0.35 for both sexes and areas as an average of mortality values estimated with different methods involving the Von Bertalanffy growth function through the Barefoot ecology toolbox (Table 5.11; Prince, 2003). A recruitment index was estimated within the model separately for each area (ITA & CRO). The ogive for the proportion at mature length in "Pomo" area was estimated from the most recent data by Angelini et al. (2020) as well as the length-weight relationship (Table 5.11).

Table 5.11. Summary by sex of biological key input parameters. The growth rate "*growth"* was expressed by Francis parametrization; "L" indicates the lower size (expressed as CL in mm) bound of the size class < i > (here defined as α and β), while "g" is the growth increment at size < i > . The "*maturity_props.all"* reported the size at first maturity in term of carapace length (CL$_{50}$) with the related time (T$_{L50}$) expressed as age-classes for both sexes used to build the maturity's ogive. The size weight relationship were calculated using the formula: W=aL^b (Pauly, 1984) where 'a' is an intercept and 'b' is the power function. The natural mortality value fixed for both sexes were also reported.

	source	***males***	***females***
growth	from Froglia and Gramitto (1988)	L_α = 16.69 L_β = 39.45	L_α = 16.05 L_β = 33.9

		g_α = 10.13 g_β = 3.834	g_α = 9.2 g_β = 1.887
maturity_props.all	from Angelini et al. (2020)	CL_{50} = 24.332 T_{L50} = 1.722459	CL_{50} = 21.14 T_{L50} = 1.48746
Size_weight relationship	from Angelini et al. (2020)	a = 0.0000000003 b = 3.203	a = 0.0000000013 b = 2.762
natural_mortality	from Barefoot ecology toolbox (Prince, 2003)	Fixed at 0.35	

Commercial fishery and survey selectivities were assumed to be the same in the two areas but varied between time steps and surveys (Table 5.12). Basically, for both Italian and Croatian fishery selectivities in TS2 (April to July) a logistic-capped ogive was selected for both sexes because theoretically all sexes and ages are equally available by fishery during the spring-early summer season (peak of emergence due to the mating behaviour; Aguzzi et al., 2004b, 2007); the same selectivity ogive was thus settled for commercial fisheries, UWTV trawl and camera indices. In TS1 (January to March) and TS3 (August to December) a logistic-capped ogive for males and a double normal-caped one for females were selected as commercial fishery selectivities allowing for differences in overall catchability between the sexes. The reasons are based on the behaviour of the species accounting for cyclical emergency from burrows of adult males due to the establishment of hierarchies of dominance (Sbragaglia et al., 2017); by contrast mature (larger) females tend to stay longer in burrows while ovigerous during autumn-winter season thus influencing the sex ratio proportion within commercial catches (Aguzzi *et al.*, 2007). Furthermore, the length frequency data showed evidence that the ScamPo survey was not catching large males (which were caught by the commercial fishery), implying reduced availability to the survey (potentially related to spatial targeting by the fishery). A double normal selectivity was therefore also applied for males in the ScamPo survey.

Table 5.12. Fitted and estimated selectivity parameters in each fishery/survey by time step for both sexes. Please note that selectivity for TS1 is the same of TS3. For a description of the parameters used to set the ogives please see the CASAL manual in Bull et al. (2012). The asterisk (*) refers to fixed values.

Selectivity name	***Description***	***selectivity ogives***	***Fitted parameters***	***Estimated parameters***

POM_1_ITA	Italian Fishery selectivity, step 1 and 3	*Males* logistic_capped	a_{50} = 30 a_{to95} = 5 a_{max} = 1	a_{50} = 24.1773 a_{to95} = 6.42756 a_{max} = 1.0964
		Females double_normal_capped	a_{1} = 30 s_{L} = 5 s_{R} = 15 a_{max} = 1	a_{1} = 29.4677 s_{L} = 5.71879 s_{R} = 17.6114 a_{max} = 1.10182
POM_2_ITA	Italian Fishery selectivity, step 2	*Males* logistic_capped	a_{50} = 30 a_{to95} = 5 a_{max} = 1	a_{50} = 28.85 a_{to95} = 12.8661 a_{max} = 0.843184
		Females logistic_capped	a_{50} = 30 a_{to95} = 5 a_{max} = 1	a_{50} = 28.815 a_{to95} = 12.5256 a_{max} = 1.34623
POM_1_CRO	Croatian Fishery selectivity, step 1 and 3	*Males* logistic_capped	a_{50} = 30 a_{to95} = 5 a_{max} = 1	a_{50} = 22.822 a_{to95} = 4.39898 a_{max} = 0.713196
		Females double_normal_capped	a_{1} = 30 s_{L} = 5 s_{R} = 15 a_{max} = 1	a_{1} = 29.447 s_{L} = 4.54425 s_{R} = 8.74905 a_{max} = 1.31743
POM_2_CRO	Croatian Fishery selectivity, step 2	*Males* logistic_capped	a_{50} = 30 a_{to95} = 5 a_{max} = 1	a_{50} = 23.248 a_{to95} = 4.06939 a_{max} = 0.894498
		Females logistic_capped	a_{50} = 30 a_{to95} = 5 a_{max} = 1	a_{50} = 25.322 a_{to95} = 5.28345 a_{max} = 1.11877
UWTV	UWTV trawl survey selectivity, step 3	*Males* logistic_capped	a_{50} = 20 a_{to95} = 5 a_{max} = 1	a_{50} = 13.2743 a_{to95} = 2.69893 a_{max} = 0.723989
		Females logistic_capped	a_{50} = 20 a_{to95} = 5 a_{max} = 1	a_{50} = 14.8329 a_{to95} = 6.78353 a_{max} = 1*
ScamPo	ScamPo trawl survey selectivity, step 2	*Males* double_normal	a_{1} = 20 s_{L} = 5 s_{R} = 10	a_{1} = 28.6066 s_{L} = 8.15953 s_{R} = 43.4461
		Females double_normal	a_{1} = 20 s_{L} = 5 s_{R} = 10	a_{1} = 24.233 s_{L} = 6.10504 s_{R} = 5.26957
I-UWTV	Camera survey selectivity	logistic_capped	a_{50} = 25 a_{to95} = 5 a_{max} = 1	a_{50} = 25 a_{to95} = 5 a_{max} = 1*

Fits to the 2 stock “Pomo” model are presented below, with key parameter estimates provided in table 5.13. CASAL allows to calculate the Spawning Stock Biomass (SSB) for each stock as the mature biomass (of both sexes) halfway through the natural and fishing mortality in each time step settled. The model estimated SSB_0 (the “0” refers to the virgin biomass named B_0) for the “Pomo” ITA stock of 12700 tonnes, with SSB_{2019} estimated at 306 tonnes, 2.5% of SSB_0; while for the “Pomo” CRO stock the model estimated SSB_0 of 2700 tonnes, with SSB_{2019} estimated at 653 tonnes, 25% of SSB_0. The model fitted the survey indices quite well with the exception of I-UWTV data which tends to overestimate the camera survey data (I-UWTV) for ITA stock in the first years, while it tends to underestimate the CRO stock (Figure 5.31). The pattern of biomass estimated by the model is clearly decreasing underlining a marked decline since ‘80s (Figure 5.32). The SSB reached null value (i.e. zero biomass) in 2010 describing a probable collapse of the ITA stock, only in the last decade a slight increment was observed for CRO stock (Figure 5.32). The estimated annual recruitment (i.e. Year Class Strength, YCS) for both stocks was historically under 1 (Figure 5.33), which is intended as the equilibrium state, suggesting that the recruitment strength was never enough to replace the number of fish which were removed from the partition by the mortality (fishing + natural mortality). This result is in accord with the continuous decreasing trend estimated by the model for biomass. Following an extremely high capture event observed in 2010 at low YCS values (Figure 5.33) which this could have caused the collapse of the ITA stock, the YCS exceeds the value of 1 suggesting a possible slight recover of the biomass, at least conceivable for CRO stock. Estimated selectivities (Table 5.11; Figure 5.34) follow expected patterns, in that females availability was higher than males during time step 2. Average estimated proportions at length fitted well the average observed length distributions for both survey data (Figure 5.35) and commercial ones (Figure 5.36); the same good fit of the estimates resulted for the observed individual survey time step distributions (Figure 5.37 to Figure 5.39), but fits to individual commercial time step distributions were more variable (Figure 5.40 to Figure 5.45). However, the good adaptation of the model to the data provided encourages this model setting scheme which therefore seems to be able to catch the enormous variability of the distributions of the data used quite well. However, future action should be focused to perform a Monte-Carlo Markov Chain (MCMC) analysis in order to investigate the model uncertainties. The two stocks were thus afflicted by a very high exploitation (in particular for the Italian one) which had led to a constant decline of the resource over time until its probable collapse around 2010; in fact, in these years the model estimated an exploitation rate (intended as the relationship between catches and SSB) around 2.0 which was easily hypothesized to be an excessive value for the sustainability of the stock, also given the estimated values for the previous years (around 0.4 on average). However, in the last years of the time series (i.e. from 2010 onwards), probably following the establishment of fisheries management measures (the first occurred around 2015) that had limited the effort on this and other demersal resources inhabiting the Pomo/Jabuka

Pits, biomass values tended to remain stable (ITA stock) or slightly increase (CRO stock), although the exploitation rate always remained above 0.5 on average. It can therefore be concluded that the *Nephrops* in the Pomo/Jabuka Pits is in a warning status; starting from this stock assessment, further evaluations on the status of the resource should be focused on the estimation of standard reference points, currently used in stock assessment practises, in order to give more specific advice (in quantitative terms). Commonly the biological reference points refer both to the SSB (to see if the stock is overfished) and to F (to see if the stock is overfishing) as indicated within the validated Stock Assessment Forms (SAFs) by GFCM.

Table 5.13. Key estimated parameters from the 2 stock "Pomo" model. "ITA" and "CRO" are the two area (stocks) modelled. SSB is the Spawning Stock Biomass at time 0 (virgin biomass intended as B_0). "q" means the estimated catchability rate ranging from 0 to 10.

Parameter	***Estimate***
ITA_SSB_0	12757.3
ITA_SSB_{2019}	306.179
ITA_SSB_{2019} / SSB_0	0.024
CRO_SSB_0	2696.75
CRO_SSB_{2019}	653.82
CRO_SSB_{2019} / SSB_0	0.242
Survey q values	
I-UWTV	10
ScamPo	0.0989836

UWTV	0.0686962

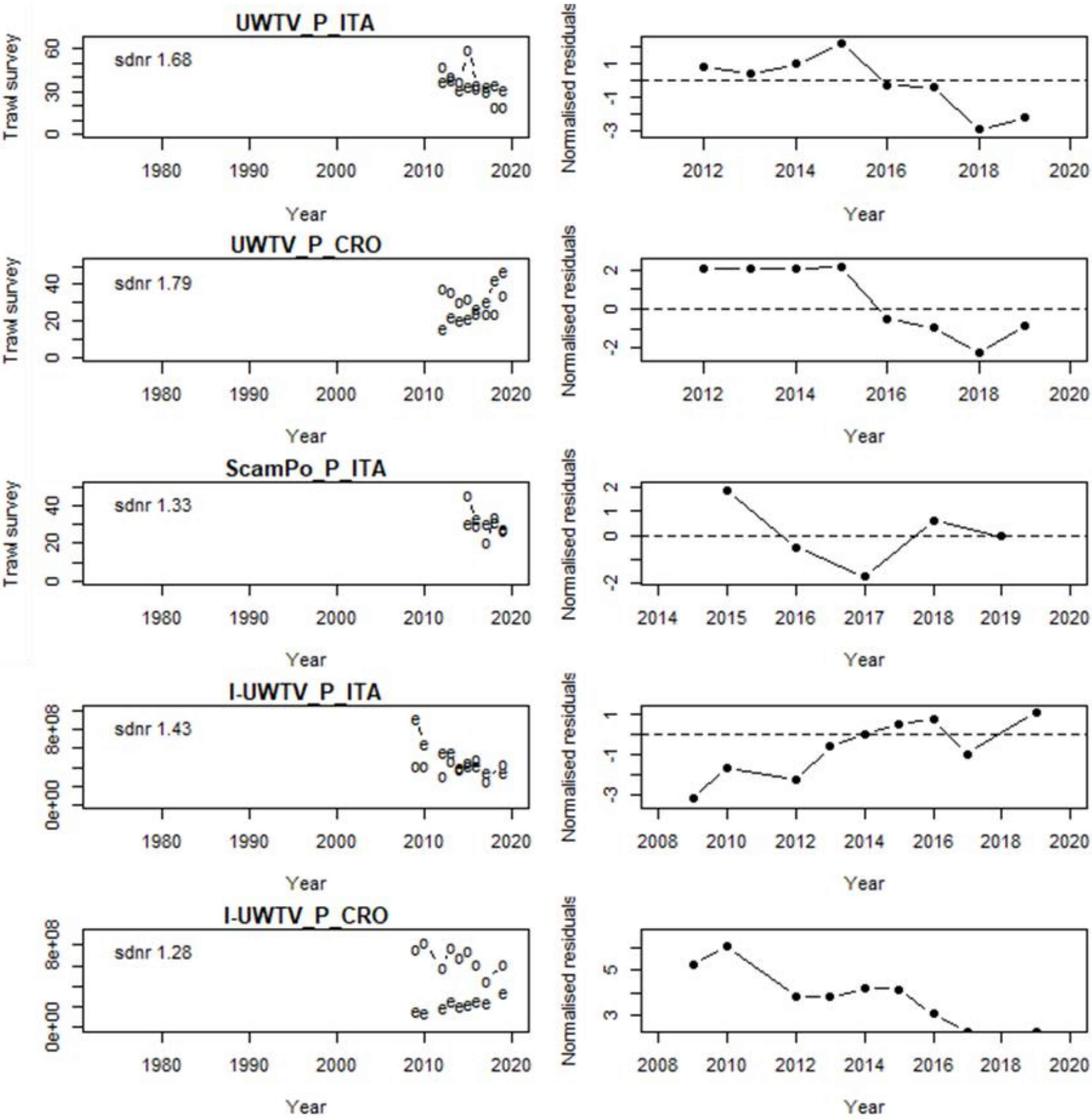


Figure 5.31. Survey input indices against model estimates by area. Fits to trawl (kg/km^2) and camera (absolute number of individuals) survey indices (left column) and normalised residuals (right column) were plotted for each survey data input (UWTV, ScamPo and I-UWTV) by area (stock). Note that camera survey indices were entered in the model by area (ITA and CRO) as well as trawl data (UWTV), apart from ScamPo survey which were performed only on the Italian side.

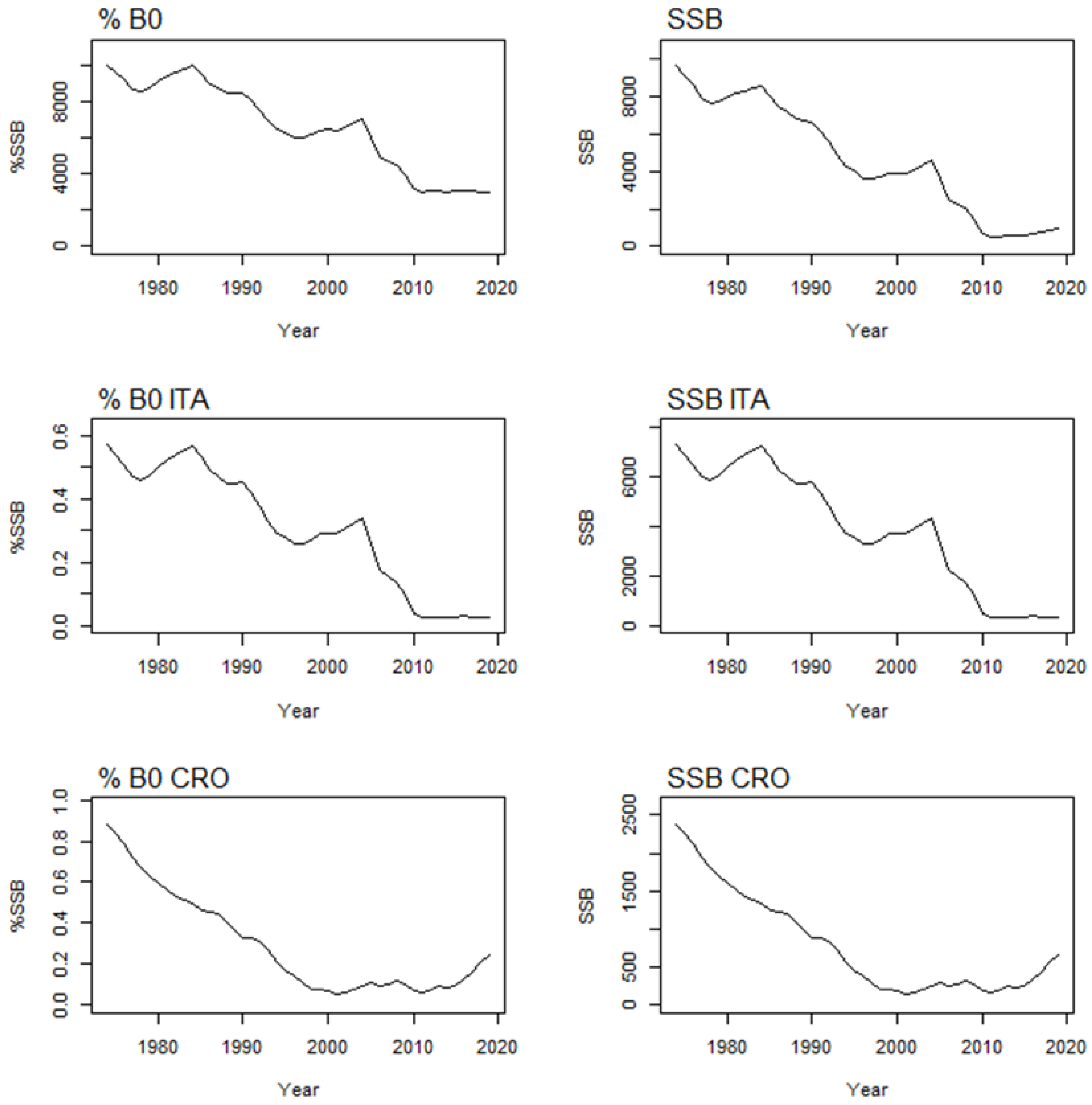


Figure 5.32. Model outputs 1. Trajectory of estimated Spawning Stock Biomass (SSB) expressed in tonnes (right column) and SSB as a percentage of SSB_0 expressed as decimal number (left column). The SSB are showed as combined (top panels) as well as for both Italian (middle panels) and Croatian (bottom panels) areas (stocks).

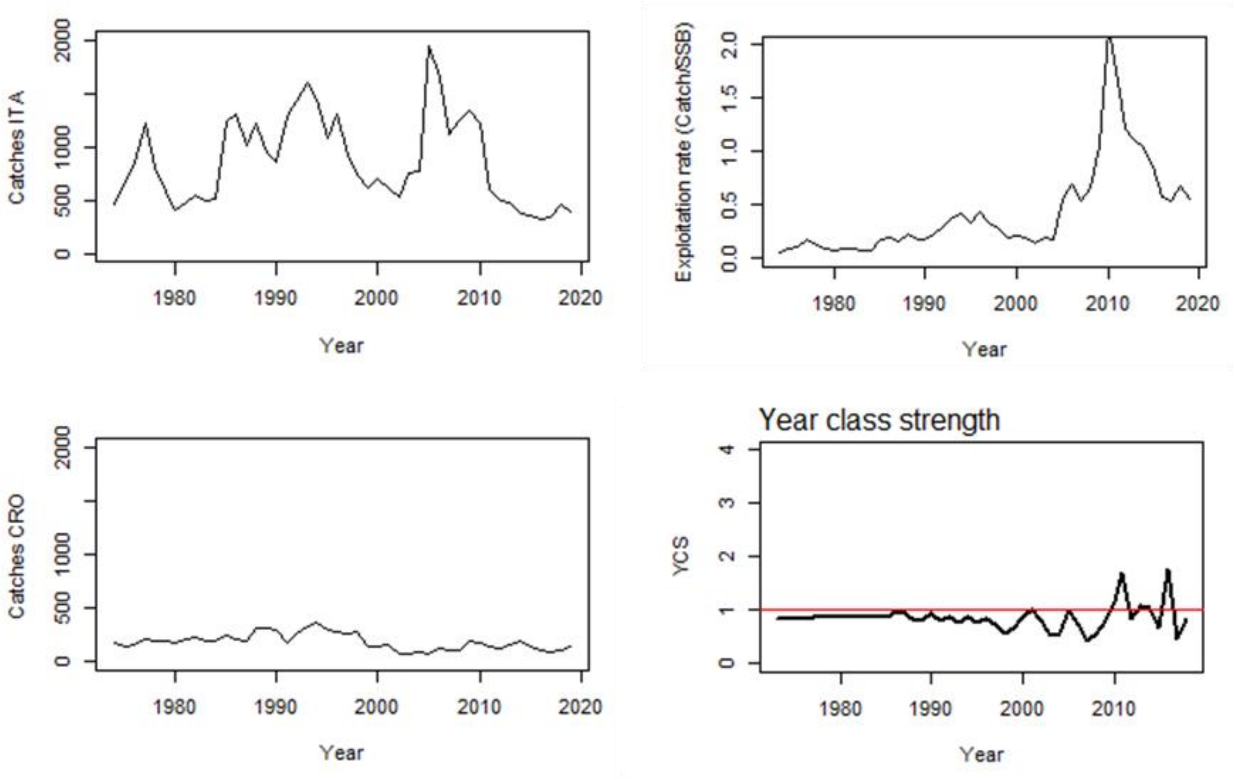


Figure 5.33. Model outputs 2. Catches for ITA stock (top left) and CRO stock (bottom left), exploitation rate intended as Catch/SSB (top right) and trajectory of Year class strength (bottom right) estimated for ITA and CRO stocks combined. Note that YCS = 1 represents the equilibrium state between recruited and removed individuals from the partitions.

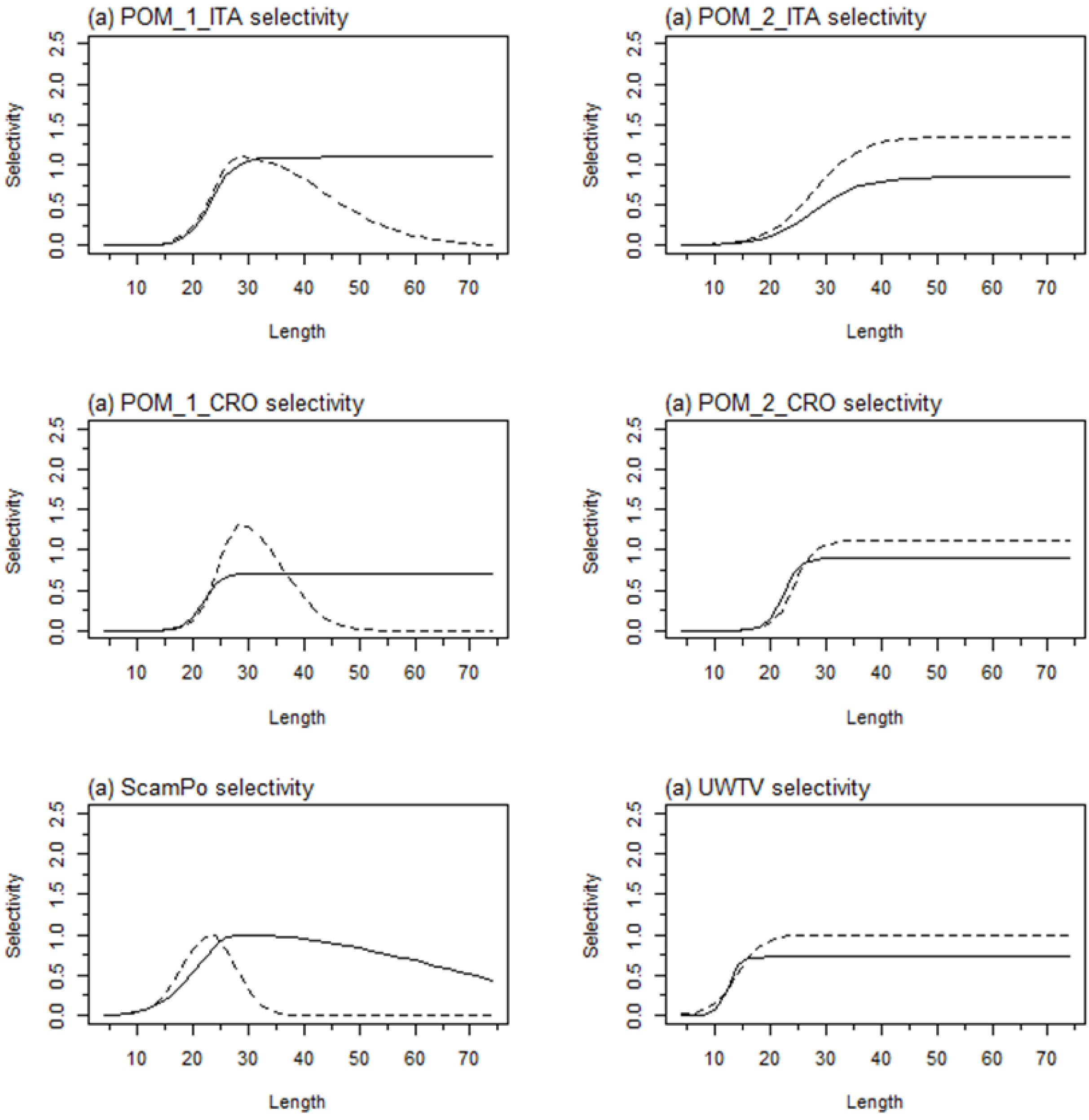


Figure 5.34. Fishery and survey estimated selectivity curves. The selectivity curves applied to TS1 and TS3 (left panels) and the ones applied to TS2 (right panels) were estimated for both fisheries (POM_ITA and POM_CRO) and surveys (ScamPo and UWTV) and here plotted by sex (Solid line – males, dotted line – females). Note that the camera survey (I-UWTV) was not displayed because the selectivity ogive values were fixed (see Table 5.11).

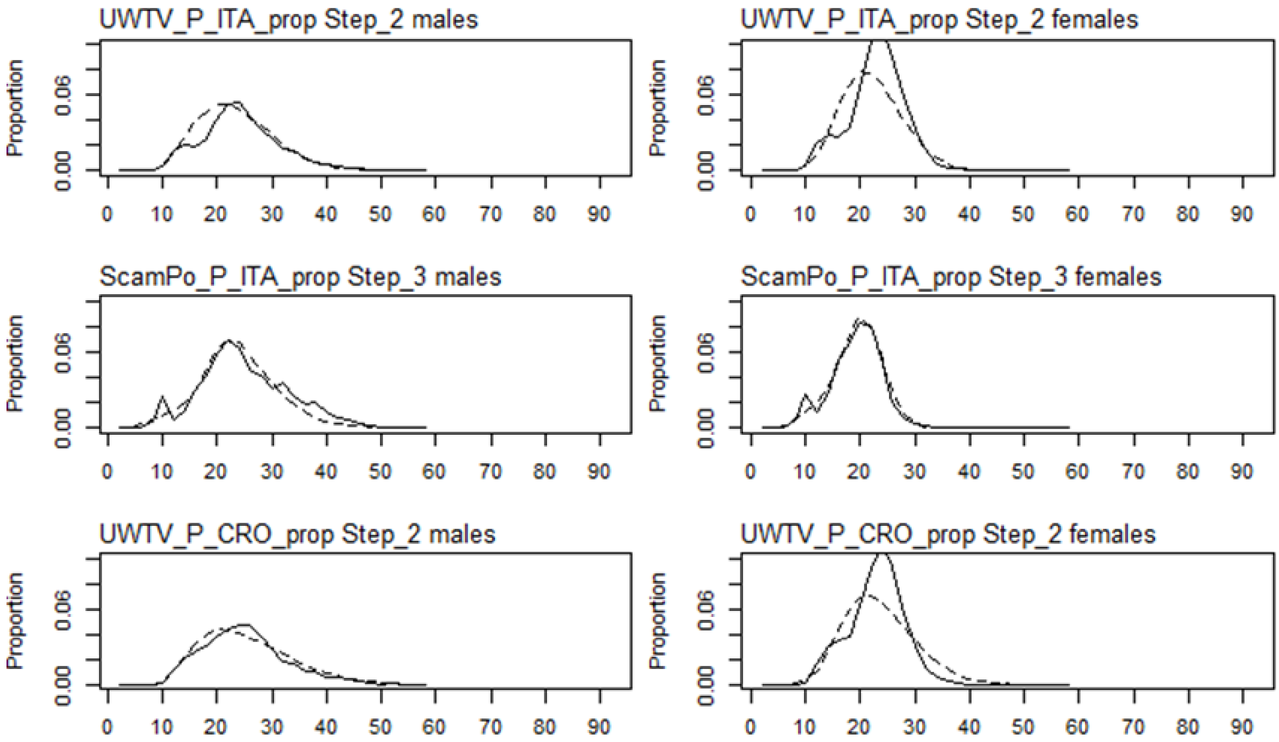


Figure 5.35. Average proportions at length across all sampled years for survey data, compared to estimated. The average observed (solid line) and estimated (dashed line) proportions of the length frequency distributions were showed for both UWTV (ITA and CRO) and ScamPo (only ITA) surveys by sex (males on the left, females on the right).

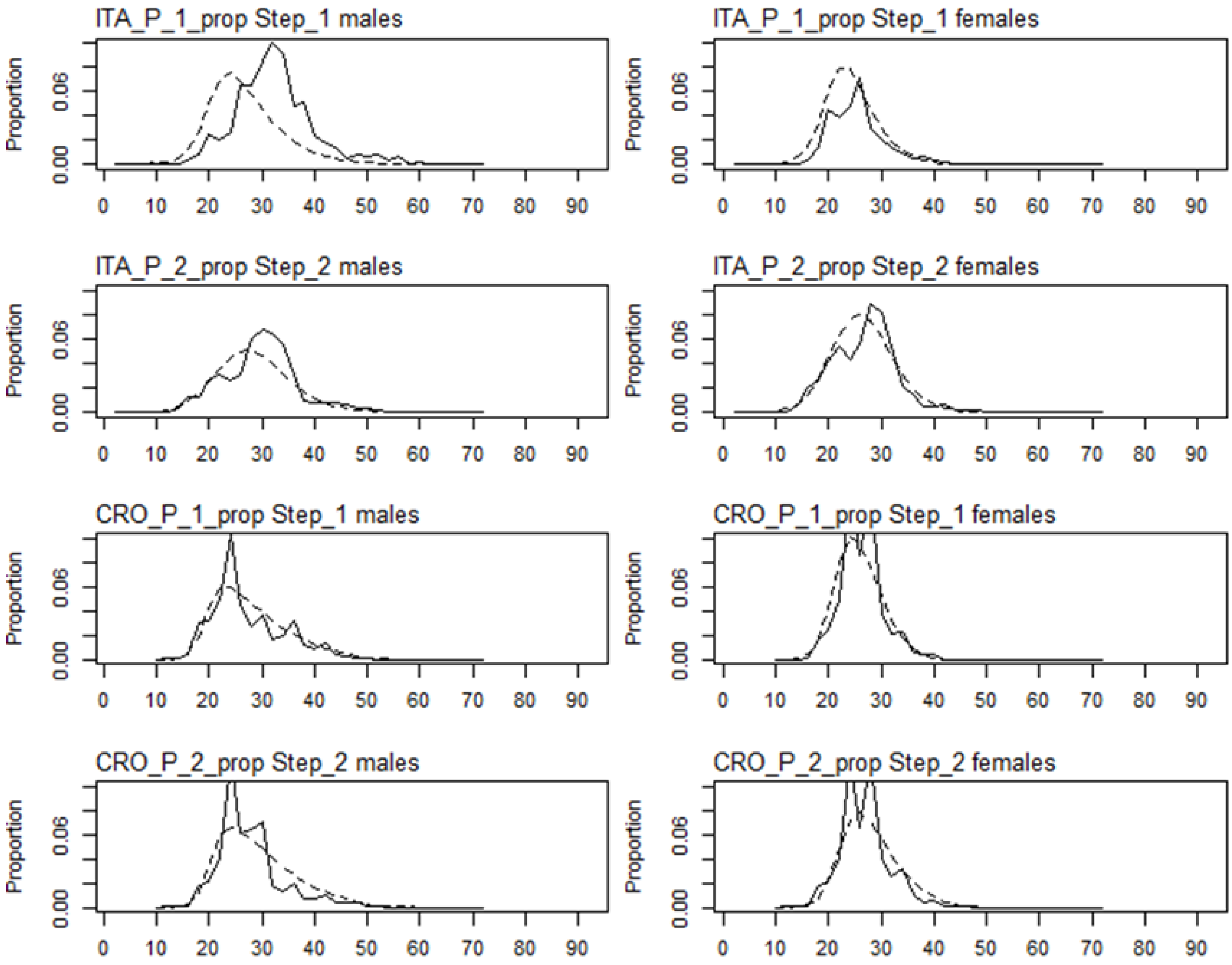


Figure 5.36. Average proportions at length across all sampled years for commercial data, compared to estimated. The average observed (solid line) and estimated (dashed line) proportions of the length frequency distributions were showed for both fisheries (ITA and CRO) and each time step (TS1 and TS3 were aggregated) by sex (males on the left, females on the right).

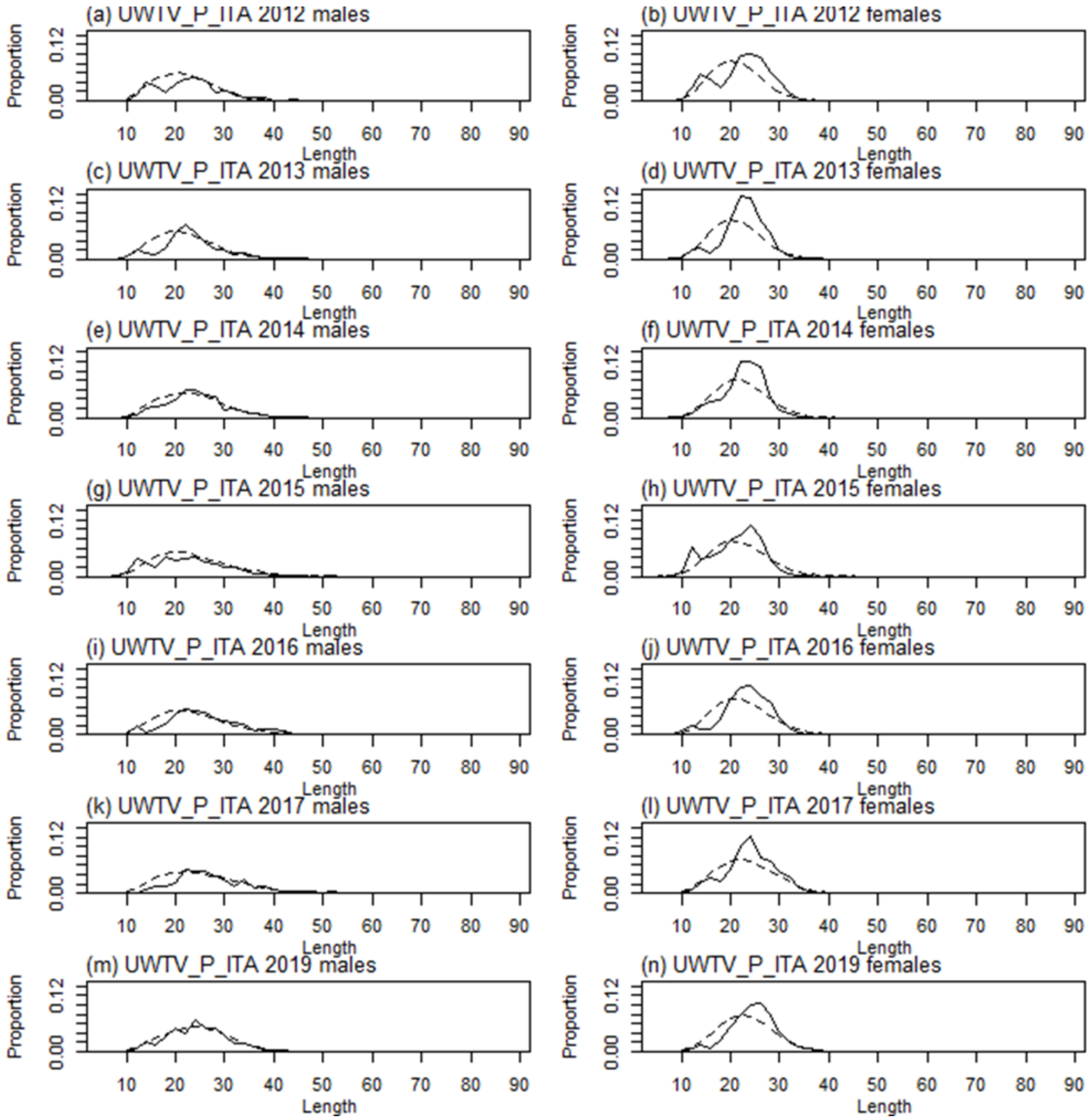


Figure 5.37. Proportions at length by year for UWTV survey data (ITA), compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed for the only Italian area (stock) by sex (males on the left, females on the right).

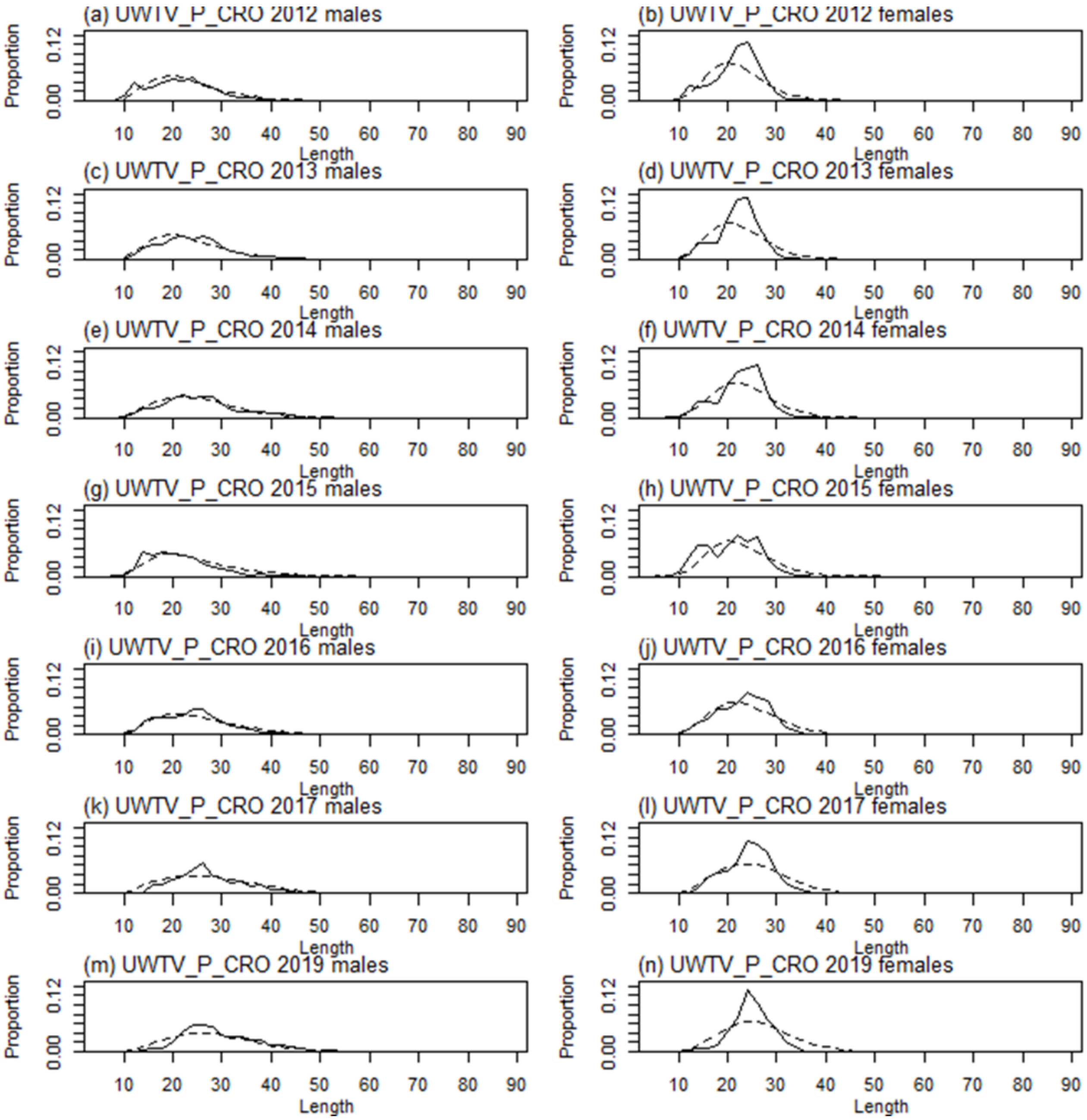


Figure 5.38. Proportions at length by year for UWTV survey data (CRO), compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed for the only Croatian area (stock) by sex (males on the left, females on the right).

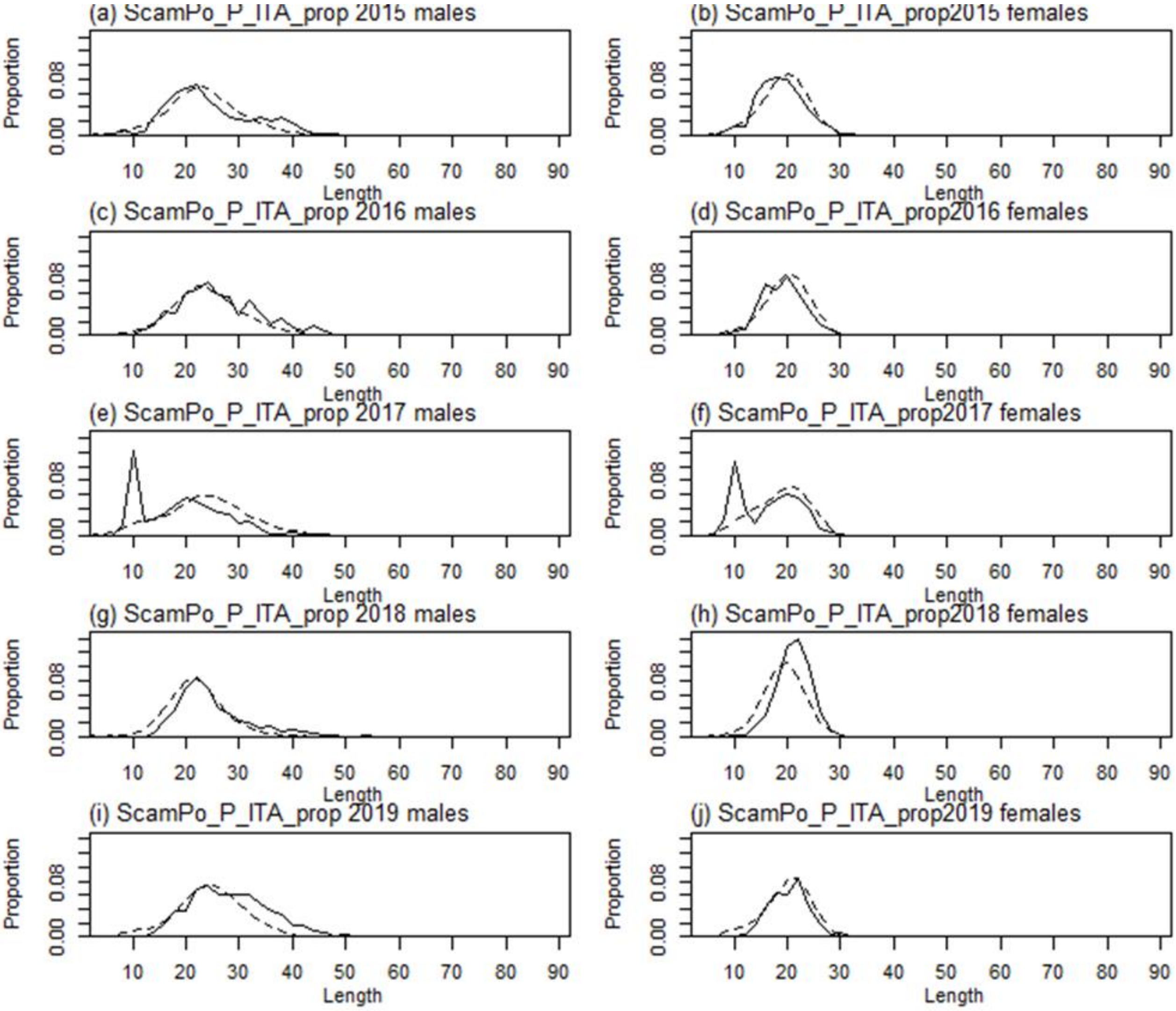


Figure 5.39. Proportions at length by year for ScamPo survey data, compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed by sex (males on the left, females on the right).

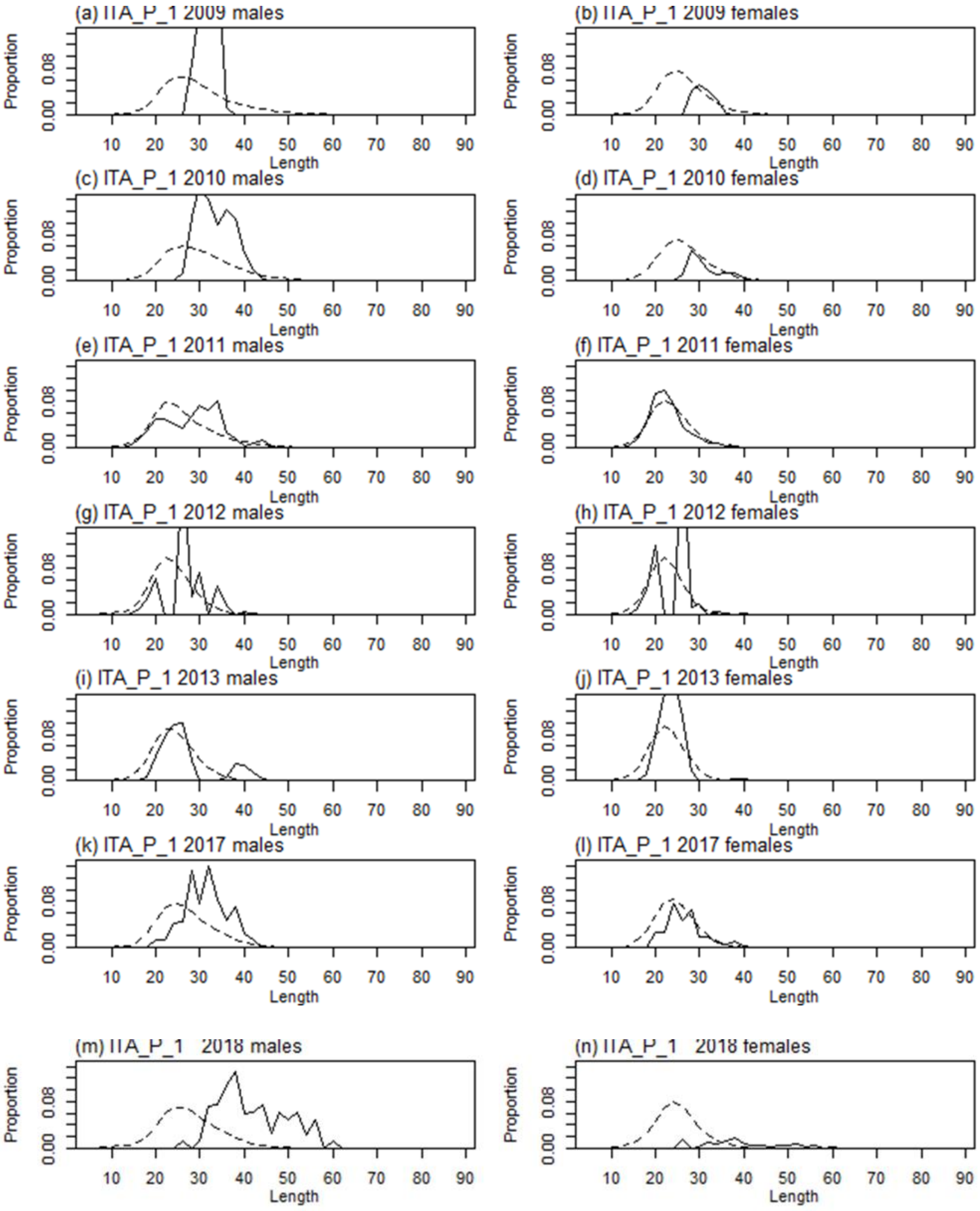
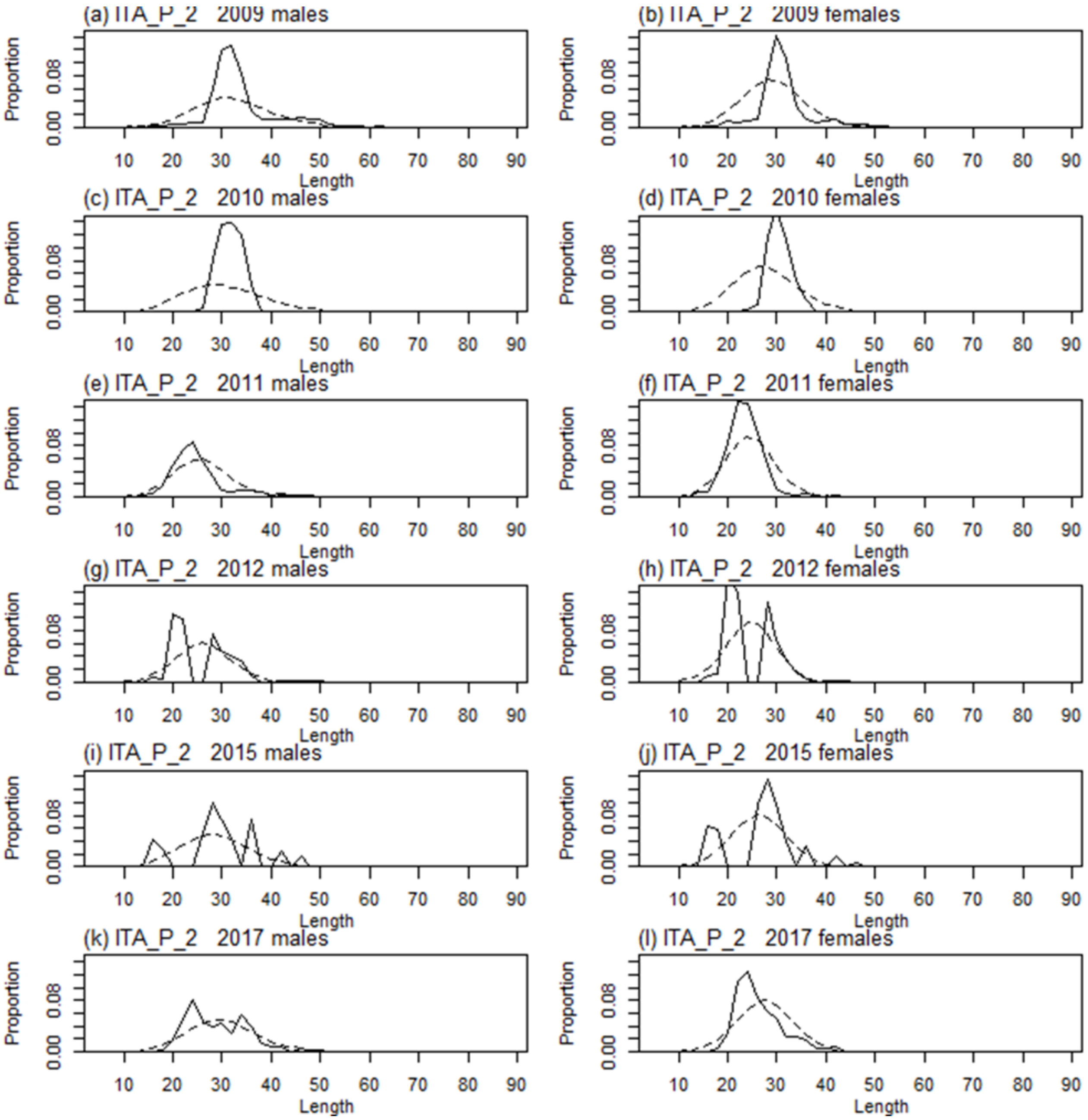


Figure 5.40. Proportions at length by year for Italian commercial data in TS1, compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed by sex (males on the left, females on the right).


(a) ITA_P_2 2009 males
(b) ITA_P_2 2009 females
(c) ITA_P_2 2010 males
(d) ITA_P_2 2010 females
(e) ITA_P_2 2011 males
(f) ITA_P_2 2011 females
(g) ITA_P_2 2012 males
(h) ITA_P_2 2012 females
(i) ITA_P_2 2015 males
(j) ITA_P_2 2015 females
(k) ITA_P_2 2017 males
(l) ITA_P_2 2017 females
Proportion
0.00
0.08
10
20
30
40
50
60
70
80
90
Length

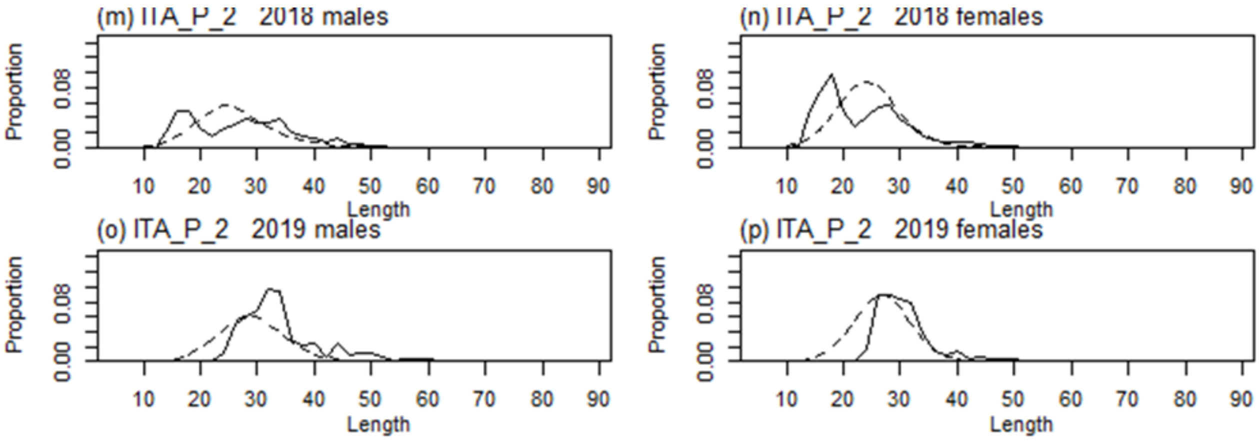
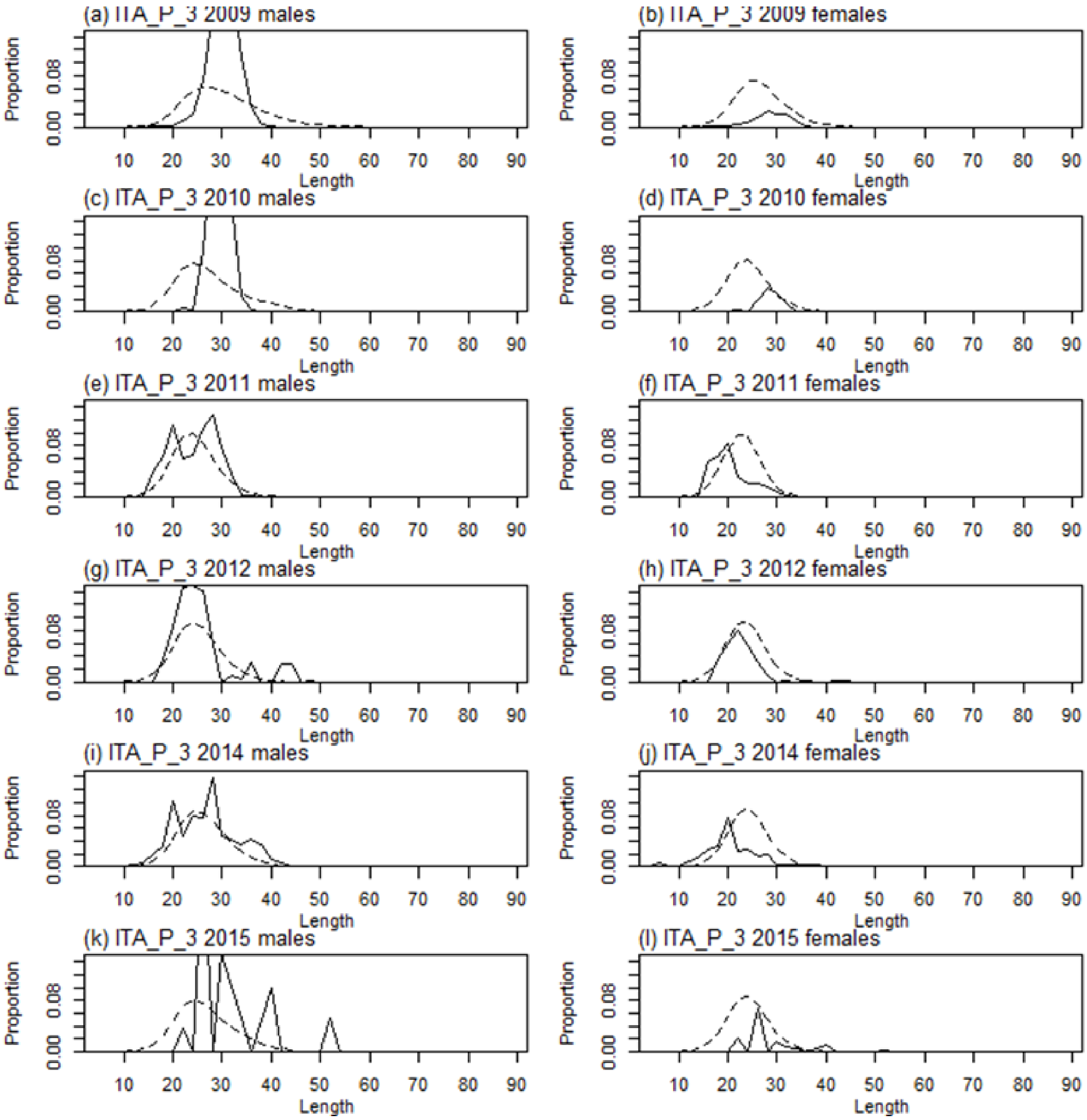


Figure 5.41. Proportions at length by year for Italian commercial data in TS2, compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed by sex (males on the left, females on the right).


(a) ITA_P_3 2009 males
(b) ITA_P_3 2009 females
(c) ITA_P_3 2010 males
(d) ITA_P_3 2010 females
(e) ITA_P_3 2011 males
(f) ITA_P_3 2011 females
(g) ITA_P_3 2012 males
(h) ITA_P_3 2012 females
(i) ITA_P_3 2014 males
(j) ITA_P_3 2014 females
(k) ITA_P_3 2015 males
(l) ITA_P_3 2015 females
Proportion
0.00
0.08
Length
10
20
30
40
50
60
70
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90

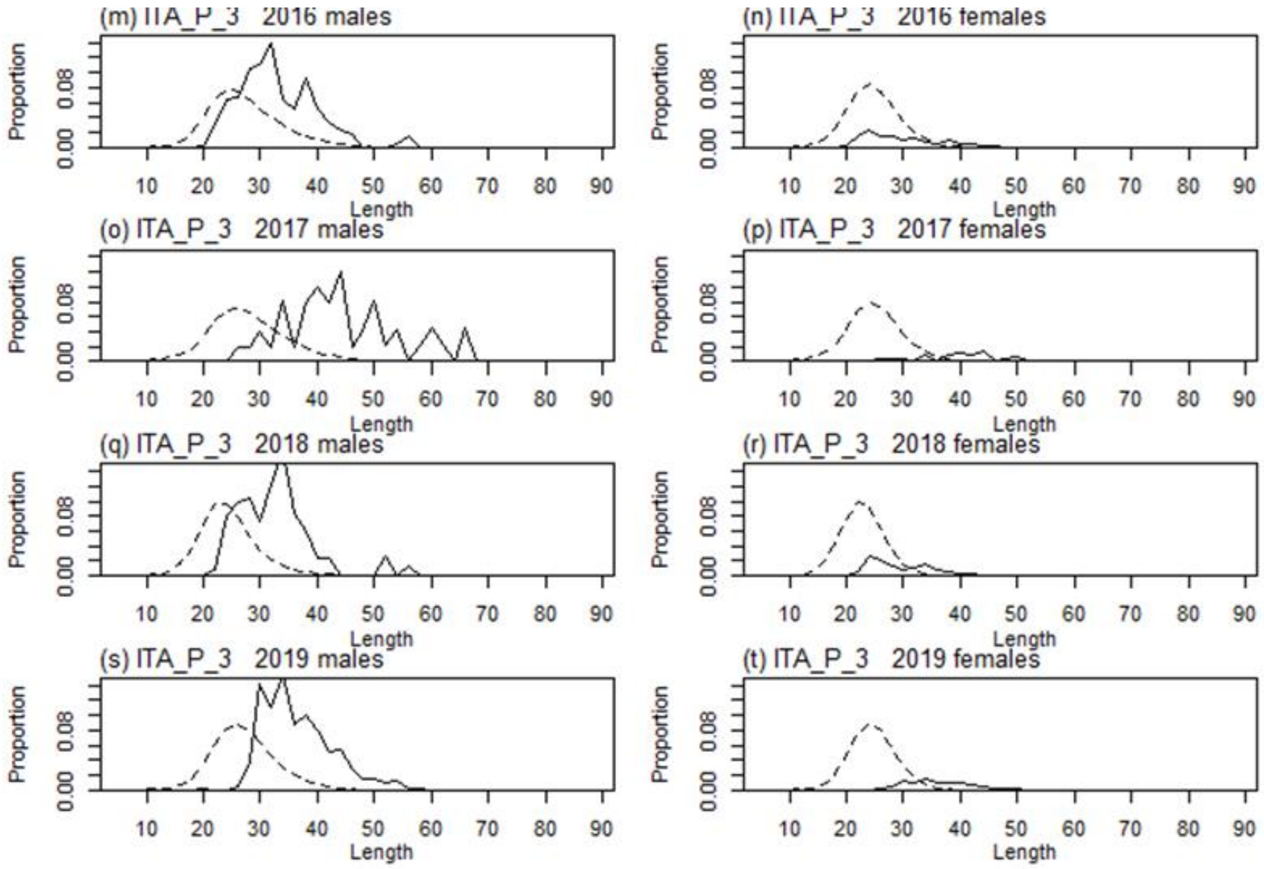

Figure 5.42. Proportions at length by year for Italian commercial data in TS3, compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed by sex (males on the left, females on the right).

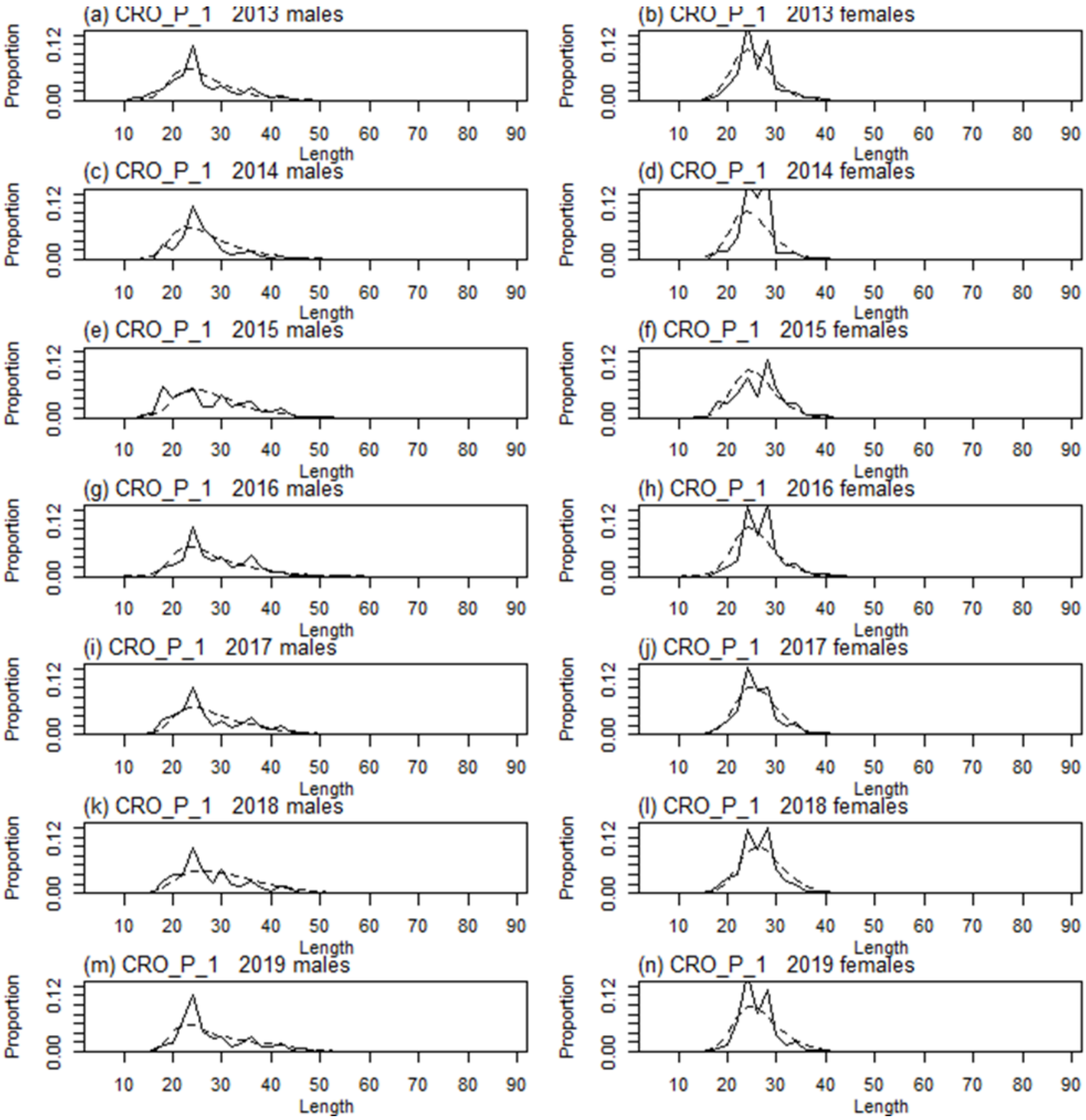

Figure 5.43. Proportions at length by year for Croatian commercial data in TS1, compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed by sex (males on the left, females on the right).

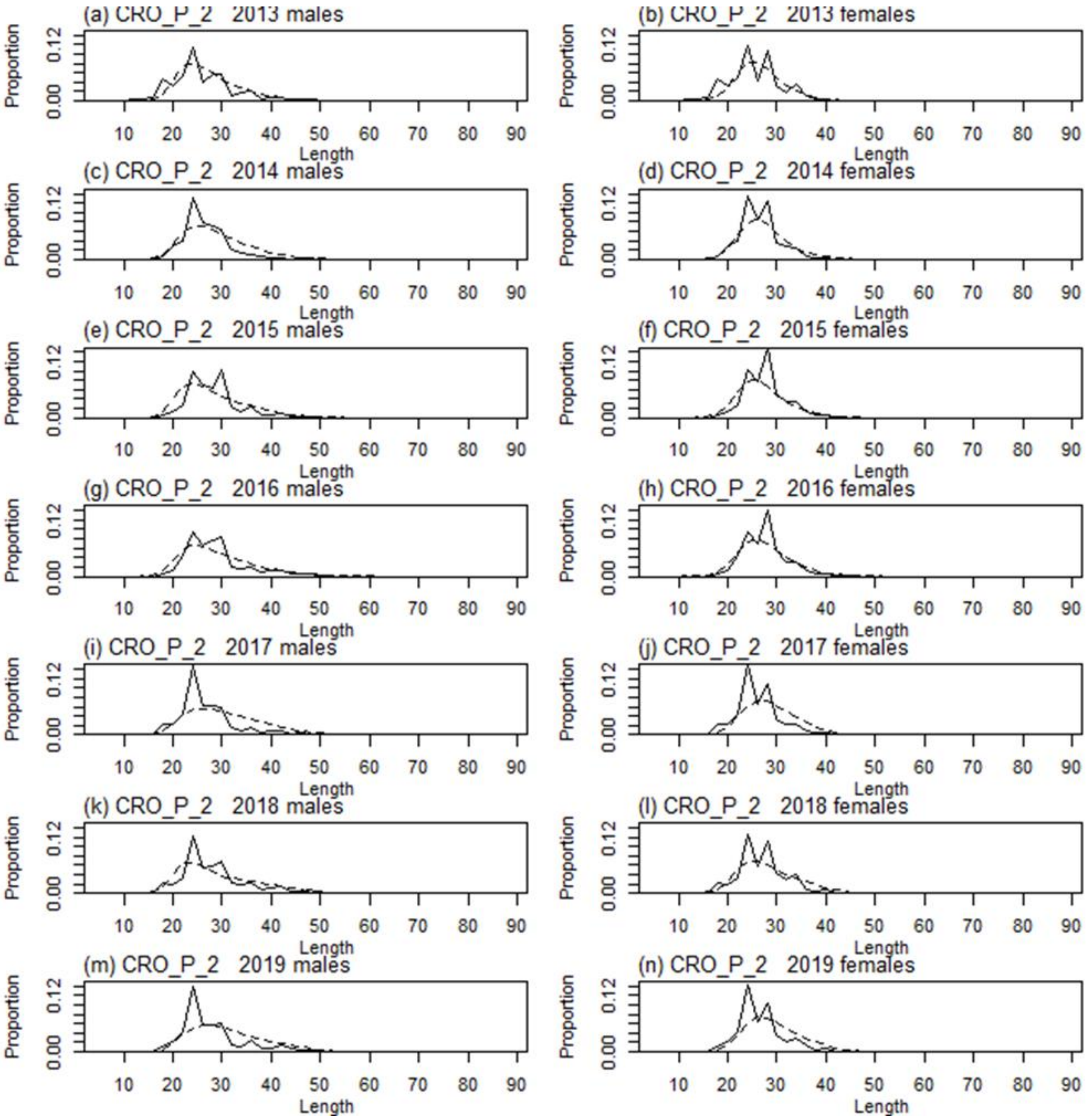

Figure 5.44. Proportions at length by year for Croatian commercial data in TS2, compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed by sex (males on the left, females on the right).

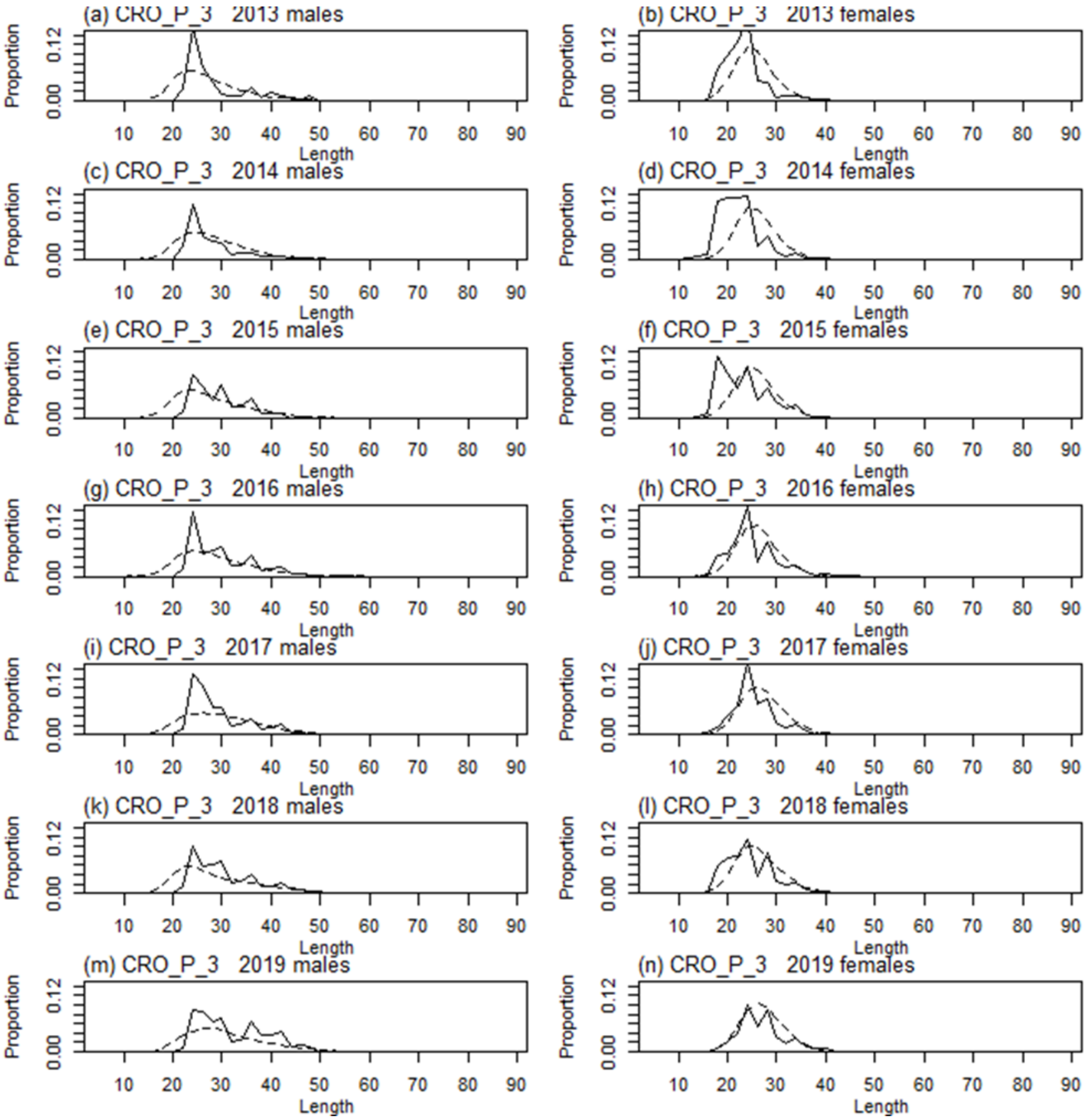

Figure 5.45. Proportions at length by year for Croatian commercial data in TS3, compared to estimated. The observed (solid line) and estimated (dashed line) proportions of the available length frequency distributions were showed by sex (males on the left, females on the right).

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6. Conclusions

This study assesses for the first time the status of *Nephrops* within an ecologically and commercially important area of the Central Adriatic Sea, the Pomo/Jabuka Pits. In fact, the main criticisms attributable to *Nephrops* assessments presented for the Adriatic Sea until nowadays were: i) a too simple characterization of the biology and behaviour of the species itself within the scientific and commercial input data, ii) the lack of inclusion of different population structure among *Nephrops*' grounds in the population dynamic model (in particular of the different growth rates and length compositions), iii) the absence of spatially explicit commercial catch data at a local scale (e.g. the "Pomo" study area).

To overcome the listed issues, within this study and in order to be as more accurate as possible, a series of data input never included before in an official stock assessment were refined and taken into consideration: (i) the absolute numbers of individuals derived from burrow density estimates obtained by video analysis of seabed footage collected during the UWTV surveys; (ii) CPUE indices from two seasonal trawl surveys ("UWTV" & "ScamPo") designed to specifically target *Nephrops* and standardised for both environmental and fishery management effects; (iii) landings and length composition of the commercial catches by country collected within the Data Collection Framework (DCF) and tuned for the study area accordingly to the official statistic on productions. The assessment was based on a Bayesian length-based model (i.e. CASAL) flexible enough to assess the peculiar biology of *Nephrops* and its fishery patterns.

Given the number of issues underlying this assessment, the UWTV footage time series is meant to provide good estimates on the absolute numbers of individuals to be entered as an input in the assessment model. Therefore, the CNR-IRBIM burrow density time series for the period 2012-2017 was reanalyzed (extensively applying the Lin's Coefficient of Concordance to burrow readings) in order to obtain reliable estimates of the absolute numbers of individuals inhabiting the "Pomo" study area. The review of the time series from 2012 to 2017 validated 93% of readable stations. New readings for 2019 were carried out accordingly and about 70% of readable stations passed Lin's CCC test. Lin's CCC proved to be a reliable statistical method for the evaluation of the homogeneity of the readings between counters; it is then strongly suggested to keep this protocol during future Adriatic UWTV surveys in order to statistically remove the subjectivity during the readings and the stations where the level of accordance among readers is very low (i.e. the most difficult to read). The achieved results were presented at the WGNEPS (ICES, 2020; see chapter 3.1).

UWTV methodology provides *Nephrops* abundance estimations based on the assumption "one animal = one burrow". However, stock size may be uncertain depending on true rates of burrow occupation. Thus, a joint work was carried within the ICES WGNEPS to estimate the burrow occupancy rate and to derive a ratio that could be applied when using the density index in stock assessment models (Aguzzi et al., 2021; see chapter 3.2). Thus, for the first time, UWTV footage collected around Ireland was used to investigate this uncertainty using Generalized Additive models (GAM). Comparisons of burrow densities with densities of visible animals (both animals waiting at the tunnel entrance and animals in full emergence) were analysed at time windows of expected maximum emergence. The results highlighted that on average there is about 1 visible individual per 10 burrows and suggested that this could be applied also for the Adriatic. The newly proposed burrow occupancy rate was thus applied to the estimates obtained from the Adriatic footage to be used for the assessment.

The study area (i.e. Pomo/Jabuka Pits) is characterized by a peculiar bathymetry and oceanographic regime, which make it an ideal habitat for *Nephrops norvegicus*; besides, the area is a historically highly exploited ground for Italian and Croatian fisheries. For this reason, since 2015, the Pomo/Jabuka Pits area was subject to various fishing regulations changing in type of restriction and over time and space, until the definitive establishment in 2017 of a Fishery Restricted Area (FRA). With the aim to evaluate the possible effects of changes in fishery management measures implemented in the area since 2015, a BIAMS (Before–Intermediate–After Multiple Sites) analysis was carried out on abundance indices (i.e. kg/km^2 and N/km^2) obtained from two seasonal trawl surveys ("UWTV" & "ScamPo"). The considered time series ranged from 2012 to 2019 for the spring surveys, and from 2015 to 2019 for the autumn ones (Chiarini et al., submitted; see chapter 4.1). The BIAMS was design to overcome the issue of the unavailability of a proper independent control site as well as to account for regulation changes in time. Furthermore, the adopted spatial stratification, which was a posteriori determined according to the latest regulations, allowed the inference of possible interactions among adjacent strata. Although, the effect of different management regimes on the indices for some species could be masked by possible changes in environmental conditions; hence future studies should investigate and possibly quantify the effect of the FRA according to changes in environmental conditions.

Therefore, the indices of abundance from the two trawl surveys carried out in the Pomo area were standardized through a GAM approach to obtain more accurate estimates of CPUE accounting for alterations on fishing effort as well as environmental changes over time. These two local surveys provided valuable information about the *Nephrops* subpopulation inhabiting the "Pomo" area. Indeed these surveys are specifically designed to target *Nephrops* by means of an experimental trawl net hauled at sunrise and sunset (i.e. the maximum peak of emergence for the area). The surveys also involve the use of oceanographic equipment to collect environmental information associated with each sampling haul. Hence, a grid for the "Pomo" area was built to integrate these extremely valuable environmental information by means of a kriging interpolation; together with the environmental variable each cell of the grid included a level of a "Fishery" factor describing the management measures adopted over time in the area (Chiarini et al., under review; see chapter 4.2). Through these informed grids it was possible to predict a value of CPUE with a very high spatial resolution. Prediction for both density and biomass indices were conducted for each year and survey combination, thus modelling the differences in the availability of *Nephrops* by season; it was also possible to fill the gaps within the historical time series, thus being able to provide a CPUE estimate for all the considered time frame. This methodology could be in the future also adapted to both experimental or commercial CPUE of other relevant species in sea areas where fishing restrictions have been established in order to obtain more accurate estimates and predictions.

To build an accurate stock assessment model, it is very important to define the spatial and temporal dimensions. For the Mediterranean, the official national commercial data are commonly provided and used at a GSA (Geographical Sub Area) and at the year level. Thus, a huge work on data treatment was also carried out within this study to define the proportion of official landings directly attributable to the Pomo study area, also on a correct temporal scale. The available historical time series (EU Data Collection Framework (DCF), historical time series from Fortibuoni et al. (2017) and from RECFISH research program for both Italy and Croatia, repectively) were analysed at a smaller spatial and temporal scale based on local knowledge of *Nephrops*' biology and fishing activities (i.e. rate of emergence in the Adriatic and management measures that alter fishing effort over the years, respectively). To deal with *Nephrops* seasonal emergency pattern all the annual data were allocated into three time steps: TS 1, from January to March (male emergence is dominant); TS 2, from April to July (both sex emerge equally); TS 3, from August to December (male emergence is dominant).

Hence according to Russo et al. (2018), by means of VMS data analysis and landings per harbour, the Italian Adriatic *Nephrops* official production since 2009 (see chapter 5.1) and the historical time series from 1974 (see chapter 5.3) were split by fishing ground in order to reconstruct a posteriori the spatial origin of the landings and to obtain estimates for the defined “Pomo” study area. Croatian official *Nephrops* landings since 2008 were instead available at fishing ground level thus easily matching the study area extension (see chapter 5.2); while for the Croatian historical data series collected from 1970 a ratio was applied to split the landings and obtain those related to the study area (see chapter 5.3). To take into account the differences in the availability to fisheries of the two sexes during solar year, the length frequency distributions available from DCF sampling were split by sex by means of two smoothing functions (one for Italy and another for Croatia) developed on the basis of the available biological data in order to apply a sex ratio to each size class. The time steps design was indeed confirmed by the observed sex-ratio proportion within commercial and scientific catches over years.

In order to overcome some critical issues (e.g. the lack of reliable age-determination) typically encountered while attempting to carry out *Nephrops* assessments by means of age based models, a length-based approach easily adaptable to the previously defined spatio-temporal scale (i.e. time step and “Pomo” area) of this case study was selected. Indeed, to account for complex data treatment and uncertainties in the growth rate typical of crustacean species, a flexible platform such as CASAL was selected as the best software to develop a reliable population dynamics model. A two area (CRO and ITA) Bayesian length-based model for *Nephrops* species inhabiting the “Pomo” study area was then developed; the model year starts from 1974 and ends in 2019. All the available fishery dependent and independent information on landings and local abundance described above was used as input by TS and by area for the model. The model output showed since the ‘80s a decline over time in Spawning Stock Biomass (SSB) for the Pomo subpopulation until 2010, when landings reached a minimum. The model estimated for this year an exploitation rate (intended as the relationship between catches and SSB) around 2.0; this high value could be a signal of a nonsustainable exploitation of the stock (here intended as the subpopulation or stocklet). Besides, in the following years (i.e. from 2010 onwards) the SSB values tended to remain stable (ITA area) or increase (CRO area). However, although in the latter period the exploitation rate remained above 0.5 on average, thus indicating a reduction of fishing effort, this was probably not enough to recover the abundance of the resource. Noteworthy is the fact that since 2015 fishery management measures have been implemented in the Pomo/Jabuka Pits area, limiting or influencing the distribution of the fishing effort.

The model outputs represent a warning about the status of the *Nephrops* subpopulation in the Pomo/Jabuka Pits area. This result is valuable since it account: i) for an important commercial species as *Nephrops*; ii) for a critical area shared by two different fleets (ITA and CRO); iii) it is the first time that this resource is evaluated with a comprehensive approach accounting for various ecological and demographic aspects of the species. However, this assessment could be further improved in the future including a standardization of Length Frequency Distributions (LFD) as well as the density indices derived from the *Nephrops* burrow readings, as already done for the trawl survey indices; in addiction, a Monte-Carlo Markov Chain (MCMC) analysis could be performed in order to investigate the model uncertainties. Further evaluations on the status of this resource should be focused on the estimation of biological reference points, currently used in the stock assessment practices, in order to give more specific advice (in quantitative terms). Furthermore, in the future this assessment model may be refined trough a sentivity analysis, for example developing models with different natural mortality rates. Finally, it would be important to apply such an assessment approach to the whole Adriatic including all the methodologies and information showed in this study.

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