Diclidophora merlangi (Kuhn, 1829) Krøyer, 1838 (Monogenea: Diclidophoridae) as an indicator of hydrocarbon pollution in the North Sea.

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Abstract

This study presents the results of analyses of data on infections of 2646 whiting *Merlangius merlangus* with the monogenean *Diclidophora merlangi.* All fish were caught in the North Sea and off the north coast of Scotland in 1990, 1993 and 1995. The aims were to analyse these data in relation to the locations of whiting sampling stations and oil installations active at that time, and to evaluate the results in terms of *D. merlangi* as an indicator of hydrocarbon pollution. Mean abundance of *D. merlangi* increased significantly with increasing proximity to the nearest oil field, with an accelerated rate of increase within approximately 2 km of the oil field. Age of oil field and whiting length showed no significant effect on parasite abundance, but there was a small difference between years. The results support those of previous studies in demonstrating the value of monogeneans as indicators of hydrocarbon pollution.

Keywords: *Diclidophora merlangi*, whiting, hydrocarbon pollution, North Sea

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1. Introduction

Drilling for oil and gas in the North Sea began in the 1950s, with the industry rapidly expanding and peaking at 137,099 thousand tonnes per day in 1999 (UK Department for Business, Energy & Industrial Strategy, 2019; UK Onshore Oil and Gas, 2020). Oil production increased steadily within the UK industry throughout the 1990s. Although production itself decreased from approximately 2000 onwards, many existing oil fields remained active and new fields were discovered and developed (UK Dept. for Business, Energy & Industrial Strategy, 2019).

While industrial installations such as oil rigs can have positive effects for fisheries, such as providing artificial reef habitat and by the establishment of no fishing zones around them, they may also have detrimental effects on fish and other organisms in their immediate vicinity as a result of hydrocarbon pollution. The source of most of this pollution is drill cuttings from rigs using oil-based mud, drill cuttings being the collective name for drilling mud, speciality chemicals and fragments of reservoir rock. These cuttings have significant effects on the benthic macrofauna around the rigs, the spatial extent of these effects depending on the volume of cuttings discharged and the tidal current regime in the area (Breuer et al. 2004). Early studies found effects on the benthos out to 1 km from the oil-boring platform with more severe effects found within a 500 m radius, where the benthic macrofauna had lower biodiversity and lower biomass (Gray et al., 1990). Kingston (1992) reported a transition from areas of high diversity disturbance to "background" diversity at between 750 and 1500 m from the source of contamination. Data from 19 sites across the UK sector of the North Sea were analysed by Henry et al. (2017) who found that effects on the benthos were generally limited to <1000 m from the structure, but two platforms historically drilled with oil-based mud showed effects up to 1.2 km away. The age of the oil installation may be an important factor in the spatial extent of these effects: Gates and Jones (2012) found that at 27 and 76 days after drilling commenced there were lower invertebrate megafaunal densities in an area extending >100 m from the well compared with pre-drill conditions, but three years later these densities had significantly increased. In addition to drill cuttings, contaminated water produced by routine activities of the oil industry is also discharged into the marine environment. Elevated levels of some of the contaminants in this water were clearly measurable within 2 km of the discharge point, but concentrations were relatively low and not thought to present an acute threat to biological systems (Harman et al*.,* 2009).

The effects of various pollutants on fish and their parasites have been the subjects of many reviews (Møller, 1987; Khan and Thulin, 1991; Overstreet, 1993; MacKenzie et al., 1995; Lafferty, 1997; Overstreet, 1997; Broeg et al., 1999; MacKenzie, 1999; Williams and MacKenzie, 2003; Sures, 2008; Blanar et al., 2009; Vidal-Martínez et al., 2009; Sures et al., 2017; Biswal and Chatterjee, 2020; Gilbert and Avenant-Oldewage, 2021). Aquatic pollution affects monoxenous fish ectoparasites in two ways: directly by effects on the parasites themselves, or indirectly by effects on the immune system of their hosts. Ectoparasite abundance may therefore increase or decrease depending on whether these adverse effects on the hosts' immune system are more severe than the direct effects on the parasite, or *vice versa.*

Increased levels of infection by ectoparasites, such as trichodinid ciliates and monogeneans, are often associated with exposure of their fish hosts to environmental contaminants. Blanar et al. (2009) carried out a meta-analysis of the published literature on the responses of different parasite taxa to specific contaminants and identified Monogenea as being susceptible to a variety of contaminants, including PAHs (polycyclic aromatic hydrocarbons) and PCBs (polychlorinated biphenyls), both of which are present in drill cuttings. Most studies on the effects of contaminants on monogeneans have been carried out in freshwater systems (Gilbert and Avenant-Oldewage, 2021). One of these, on the monogenean *Cichlidogyrus sclerosus* Paperna & Thurston, 1960, a gill parasite of Nile tilapia *Oreochromis niloticus* (L.) , showed a significant increase in abundance when exposed to sediments polluted with low to fairly high concentrations of PAHs and PCBs, although with a decrease at the highest concentrations (Sanchez-Ramirez et al*.,* 2007). In the marine environment, monogeneans of the genus *Gyrodactylus* Nordmann, 1832 have proved to be particularly useful indicators of hydrocarbon pollution (Khan and Kiceniuk, 1988; Marcogliese et al., 1998; Moles and Wade, 2001). The most convincing result was that of Pérez-del Olmo et al. (2007) who found that two monogenean species showed significantly higher (p=0.0001) prevalences on bogue *Boops boops* (L.) sampled from the same location before and after the *Prestige* oil-spill off Northwest Spain in 2002.

While most studies clearly show an increase in abundance of monogeneans with increasing levels of pollution, Khan and Payne (2004) and Sanchez-Ramirez et al. (2007)

found that abundance decreased at the highest levels of hydrocarbon pollution. These results, however, were based on laboratory studies and such high levels of pollution are less likely to be encountered in field studies such as the present one.

The monogenean *Diclidophora merlangi* (Kuhn, 1829) Krøyer, 1838, a gill parasite of whiting *Merlangius merlangus* (L.), was initially proposed as a potentially useful indicator of hydrocarbon pollution by MacKenzie et al. (1995). The reasons given for selecting this parasite as a potential indicator were as follows.

1. The biology and ecology of *D. merlangi* are relatively well known and there is a considerable body of information on its distribution and prevalence in the North Sea and adjacent waters (see Williams and MacKenzie, 2003).
2. Whiting are abundant and easy to examine for *D. merlangi*.
3. *Diclidophora merlangi* is considered to be host specific to whiting, although there are two reports of its occurrence on other host species, both of which appear to have been of accidental, and probably temporary, infections (Perdiguero-Alonso et al., 2006; Morsy et al., 2018).

Williams and MacKenzie (2003) referred to *D. merlangi* infection data collected in the early 1990s from the northern North Sea and off the north coast of Scotland. The aims of the present study were to reanalyse these data in relation to the locations of whiting sampling stations and of oil installations active in the North Sea at that time, and to interpret and evaluate the results in terms of *D. merlangi* as a potential indicator of hydrocarbon pollution.

2. Materials and Methods

2.1. Sampling

Whiting samples were caught in the northern North Sea and off the north coast of Scotland by demersal trawl in 1990, 1993 and 1995 during cruises of the SOAFD (now Marine Scotland) research vessel FRS *Scotia* (Fig. 1, Table 1). Whiting were either examined fresh aboard ship or frozen immediately after capture for later examination in the laboratory.

Each fish was measured from nose to tip of tail. The gill filaments were then examined for *D. merlangi* and the numbers on each fish were noted.

Oil field data were downloaded as ArcGIS shapefiles from the UK Oil and Gas Authority National Data Repository Data Centre. Data were sorted into shapefile layers by start year, so that only oil fields active during the year in question were used in distance calculations. Trawl data were also imported to ArcMap and sorted into layer files by year. ArcGIS was used to calculate the distance to the nearest active oil field for each trawl based on the ICES statistical rectangle in which the trawl occurred. Distance to the nearest oil field was used as a proxy for the amount of hydrocarbon pollution that fish at any given position would have been exposed to.

2.2. Data analyses

Parasite infection data are shown as prevalence and mean abundance. Prevalence is defined as the number of hosts infected with one or more individuals of a particular species (or taxonomic group) divided by the number of hosts examined, and mean abundance as the total number of parasite individuals found in a sample divided by the total number of hosts examined (Bush et al*.,* 1997). Due to the large number of possible explanatory variables, a model selection process was performed to avoid overfitting. Based on small-sample corrected Akaike information criterion (AICc), three different models were compared. Relationships between parasite mean abundance and distance to the nearest oil field, age of the nearest oil field, mean fish length, depth of oil fields and interannual variation were assessed as fixed effects using a linear mixed model (McCullagh & Nelder 1989) with location included as a random effect to better account for the complex structure and limited size of the dataset. Two reduced models were compared with the model outlined above using step-wise reduction with depth of oil field removed in one then age of oil field removed as well in the final model. The smallest model showed the lowest AICc score and largest weighting and so was selected as the final model. Model assumptions were verified by plotting residuals versus fitted values, versus each covariate in the full unreduced model, with no issues found. The model was estimated using REML and nloptwrap optimizer in the ‘lme4’ package, version 1.1-27.1 (Bates et al. 2015) in the R statistical environment version 4.1.2 (R core team 2021). The model took the form:

Parasite abundance ~ log(distance from nearest oil field) + fish length + year + (1|position), family = gaussian

Distance from oil field, fish length and year were included as fixed effects, with year as a factor with position included as a random effect to account for multiple visits to some sampling locations but only single visits to others. Distance from oil field was logged due to some skewed differences in distance.

3. Results

A total of 3309 *D. merlangi* individuals were found on the 2646 whiting examined. Whiting lengths ranged from 13 to 47 cm with a mean of 28.7 cm. Sample prevalences ranged from 40 to 100% and mean abundances from 0.4 to 2.4 (Table 2).

A linear mixed model was fitted to predict *D. merlangi* abundance, with distance to nearest oil field, fish length and year of sampling included as fixed effects. The model included position as random effect. The model's total explanatory power is substantial (conditional R^2 = 0.81) and the part related to the fixed effects alone (marginal R^2) is of 0.37 showing much of the variance is explained by differences in sampling location included as a random effect. The model's intercept, corresponding to distance to nearest oil field = 0, fish length = 0 and sampling year = 0, is at 164.55 (95% Confidence interval (CI) [46.00, 283.11], t(24) = 2.86, p = 0.009).

The effect of distance to nearest oil field is statistically significant and negative (beta = -0.23, 95% CI [-0.38, -0.08], t(24) = -3.16, p = 0.004; Std. beta = -1.48, 95% CI [-2.56, -0.40]), showing decreasing *D. merlangi* adundance with increasing distance from oil fields (Fig. 2). The effect of fish length is statistically non-significant and negative (beta = -0.03, 95% CI [-0.08, 5.93e-03], t(24) = -1.76, p = 0.091; Std. beta = -0.30, 95% CI [-0.79, 0.19]) (Fig. 3). There is a statistically significant difference between sampling years (beta = -0.08, 95% CI [-0.14, -0.02], t(24) = -2.79, p = 0.010; Std. beta = -0.32, 95% CI [-0.65, 0.02]) with 1990 having the highest abundance values for individual samples but 1993 having the highest median abundance values (Fig. 4).

4. Discussion

The present study showed a significant increase in mean abundance of *D. merlangi* with increasing proximity to the nearest oil field. The increase, however, was not gradual but showed an accelerated rate of increase within approximately 2 km of the oil field, with a levelling off beyond that distance (Fig. 3). This bears a striking similarity to the results of Gray et al. (1990), Kingston (1992) and Henry et al. (2017), all of whom found the most severe effects of hydrocarbon pollution on the benthic fauna within approximately 1 km of the nearest oilfield. Hydrocarbon pollution has been reported to impair the immune system of many species of fish and can result in higher parasite prevalences and changes in parasite faunas (Lafferty, 1997; Marcogliese et al., 1998; Moles and Wade, 2001; Sures, 2008).

One of the effects of hydrocarbon pollution on fish is an increase in numbers of mucous-producing cells in the gills, resulting in excessive mucous production (Haensley et al., 1982; Solangi and Overstreet, 1982; Khan and Kiceniuk, 1984; Nero et al., 2006; Giari et al., 2012; Wong et al., 2013). The defence ability of fish gills against parasitic infections is mainly due to the mucous layer, which contains compounds toxic for many pathogens and which can effectively target the monogenean tegument (Ilgová et al., 2021). An increase in mucous production would therefore be expected to adversely affect monogeneans, but this is apparently not the case where hydrocarbon pollution is the cause of the increase. This suggests that hydrocarbon pollution may impair the defensive nature of the mucous secretion, thereby creating a habitat more favourable for gill parasites, as suggested by Khan (1987). This is an area ripe for further research.

In a study such as this, the possible confounding effects of host age/length, host sex, season and year of sampling must be taken into consideration. Our analyses showed no significant effect of whiting length on mean abundance of *D. merlangi*, which agrees with the findings of Shotter (1973), who found no variation in either prevalence or mean intensity of *D. merlangi* in whiting from 1 to 5+ years old and with a length range of from 15.5 to 53.5 cm. The whiting in the present study were not sexed, but Shotter (1973) found no difference between the sexes in either prevalence or mean intensity of *D. merlangi.* Pilcher et al. (1989) found that mean intensities of *D. merlangi* were four times greater in summer (August – September) than in winter (February), but the possible effect of season was not a factor in the present study as all our samples were taken in the 6-week period from 26 May to 6 July. The significant ($p = 0.010$) difference recorded between mean abundances of infection between years may largely be due to the different geographical distributions of the sampling stations between years. The samples taken in 1990 were scattered over a much wider geographical area, including samples taken from off the north and far north-east coast of Scotland which were not sampled in 1993 or 1995. The much smaller number of whiting examined in 1990 compared to 1993 and 1995 is reflected in the much wider confidence limits for that year in Fig. 4. This and the general differences in mean *D. merlangi* abundance between sampling positions can be seen clearly in the differences between the conditional (0.81) and marginal (0.37) R^2 values which show that more than half of the explained variance in the model comes from the random effect, sampling position.

Another factor that must be taken into consideration is the hydrography of the study area. In depositional areas with relatively weak currents, such as the basins of the Northern and Central North Sea, drill cuttings are not rapidly dispersed but flocculate and accumulate under and around platforms (Breuer et al., 2004). Our sampling stations are spread mainly in an east-west direction from within the oil fields westwards, whereas the main inflow of water in the area is that of North Atlantic water which flows from the north-west and north southwards (Breuer et al., 2004; Sheehan et al., 2017). Water-borne hydrocarbons from the oil fields would thus be carried southwards and away from the sampling stations and so should not have had any significant effect on our results.

The results of the present study support those of previous studies in demonstrating the value of monogeneans as indicators of hydrocarbon pollution. Although earlier studies were mainly on gyrodactylids, our results show that members of the family Diclidophoridae also have serious potential as indicators. Apart from *D. merlangi*, the genus *Diclidophora* includes at least 27 other nominal species, eight of which are parasites of common and commercially important gadid fishes (Rubec and Dronen, 1994). These and other monogeneans are recommended as quick and reliable indicators of the spatial extent and severity of the effects of hydrocarbon pollution.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1. Numbers of whiting examined, with month of sampling and sampling positions (as ICES statistical rectangles). *denotes samples taken from different parts of same statistical rectangle.

Month and year	Sampling position	No. of whiting examined
May 1990	44E7	11
	46E5	20
	46E6	10
	46E7	20
	47E4	8
	47E7	20
	48E7	40
	48E9	12
	50F1	25
	51F0	37
	51F1	38
	52F0	28
1990 total		**269**
June 1993	*41E7a	450
	*41E7b	200
	45E6	200
	47E8	200
	48E8	200
	49E9	100
	49F0	32
	49F1	100
	50F0	81
	50F1	31
	51F0	200
	51F1	35
1993 total		**1829**
June 1995	41E7	100
	45E6	100
	47E8	100
	49F0	100
	50F0	100
	50F1	48
1995 total		**548**
Grand total		**2646**

Table 2. *Diclidophora merlangi* infection data and and mean whiting lengths for all samples. N = number of *D. merlangi* found, Pr(%) = prevalence of infection, MA = mean abundance. Sampling position as ICES statistical rectangles. *denotes samples taken from different parts of same statistical rectangle.

Month and year of cruise	**Sampling position**	**Mean length of whiting (cm)**	**No. of parasites**	**Pr(%)**	**MA**
May 1990	44E7	25.0	21	82.0	1.9
	46E5	30.7	16	60.0	2.0
	46E6	26.9	8	40.0	0.4
	46E7	26.7	31	80.0	1.6
	47E4	26.4	9	62.0	1.6
	47E7	25.1	25	60.0	1.2
	48E7	30.5	33	45.0	0.8
	48E9	29.1	18	58.0	1.5
	50F1	31.5	30	56.0	1.2
	51F0	31.8	69	81.0	1.9
	51F1	30.3	93	100.0	2.4
	52F0	32.6	32	61.0	1.1
June 1993	*41E7a	19.7	442	52.4	1.0
	*41E7b	20.6	205	51.5	1.0
	45E6	26.1	293	59.0	1.5
	47E8	27.8	310	66.5	1.6
	48E8	30.1	266	65.0	1.3
	49E9	33.1	143	58.0	1.4
	49F0	34.6	49	62.5	1.5
	49F1	31.9	124	56.0	1.2
	50F0	34.2	111	54.3	1.4
	50F1	33.5	40	68.0	1.3
	51F0	34.1	267	61.5	1.3
	51F1		49	68.6	1.3
June 1995	41E7	21.5	70	42.0	0.7
	45E6	22.9	89	56.0	0.9
	47E8	29.2	136	63.0	1.4
	49F0	32.0	139	63.0	1.4
	50F0	33.0	127	57.0	1.3
	50F1	31.6	64	62.5	1.3

Figure legends

Fig. 1. Chart of the study area showing ICES statistical rectangles in which samples of whiting were caught in 1990, 1993 and 1995 (see Table 1) and positions of oil fields active at the time (in red).

Fig. 2. Modelled mean *D. merlangi* abundance per fish per sample against distance to nearest oil field. Sampling took place across 3 years (1990, 1993 and 1995) with the number of fish per sample ranging from 8 to 450.

Fig. 3. Mean *Diclidophora merlangi* abundance per fish per sample by fish length for all years and sampling stations.

Fig. 4. Mean *Diclidophora merlangi* abundance per fish per sample between years for all sampling sites. Boxes represent 25-75% quartiles with the thick line representing the median. Whiskers represent the upper and lower ranges, 1.5 * the interquartile range. Dots represent outliers.

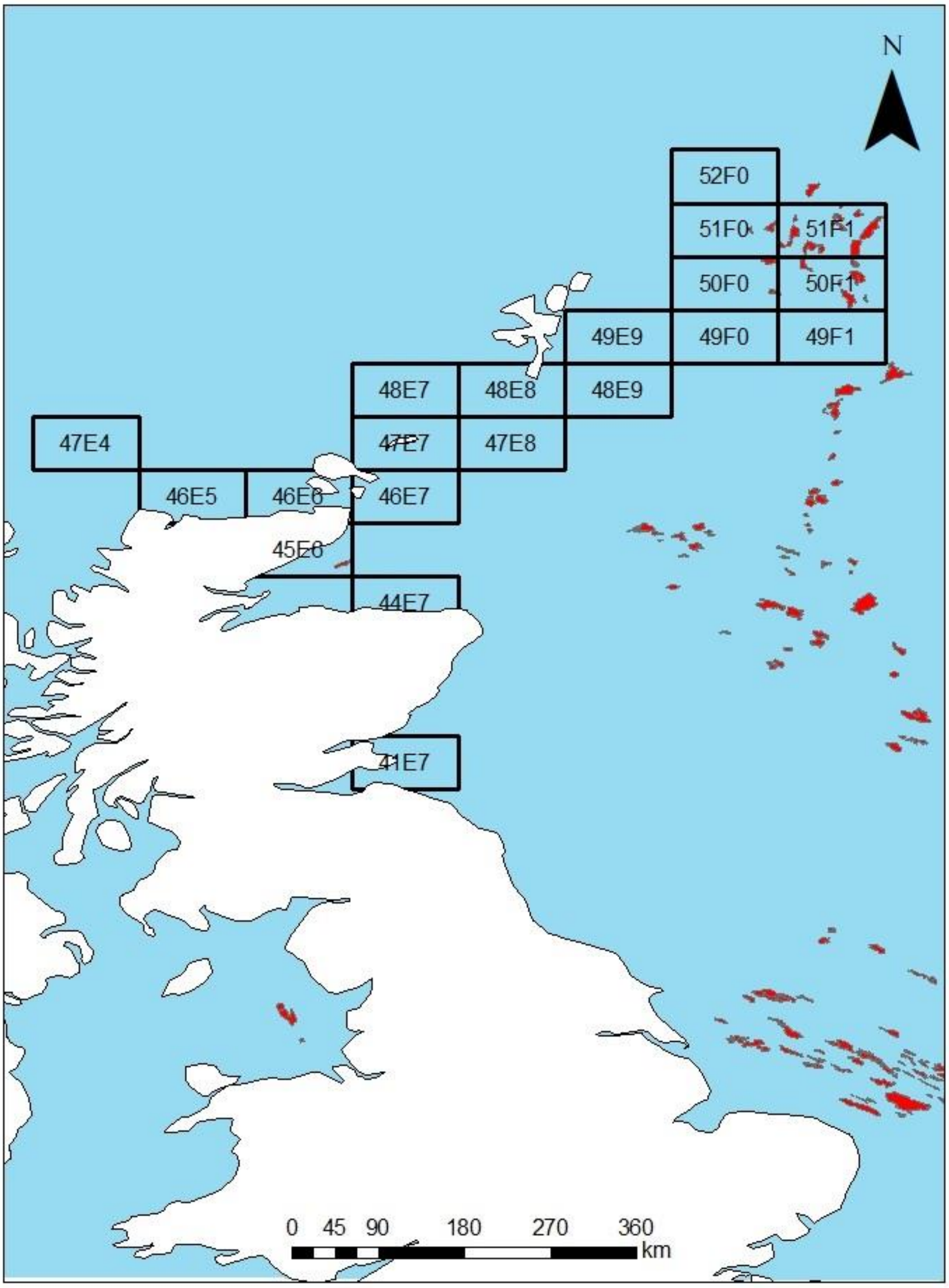

N
52F0
51F0
51F1
50F0
50F1
49E9
49F0
49F1
48E7
48E8
48E9
47E4
47E7
47E8
46E5
46E6
46E7
45E6
44E7
41E7
0 45 90 180 270 360
km

Figure 1

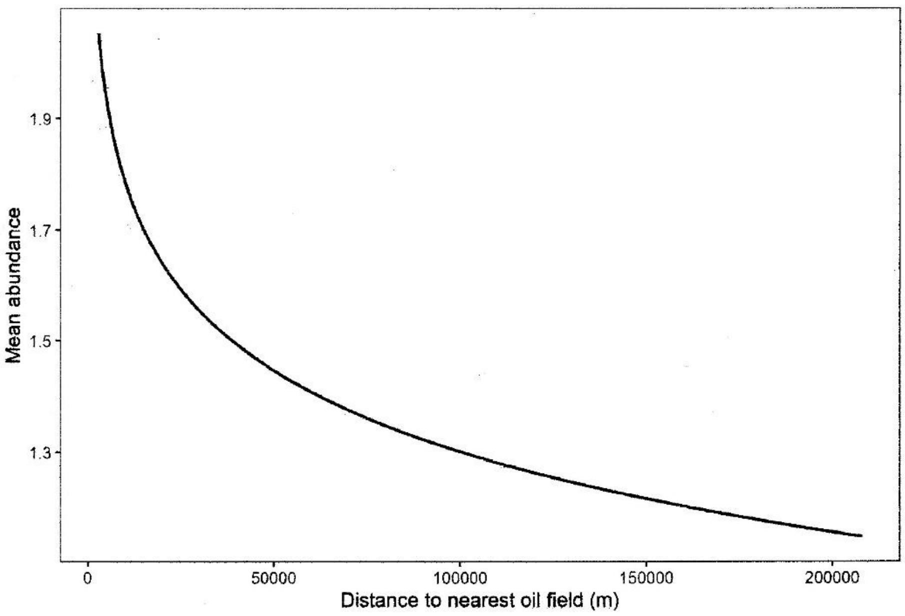

Mean abundance
1.9
1.7
1.5
1.3
0
50000
100000
150000
200000
Distance to nearest oil field (m)

Figure 2

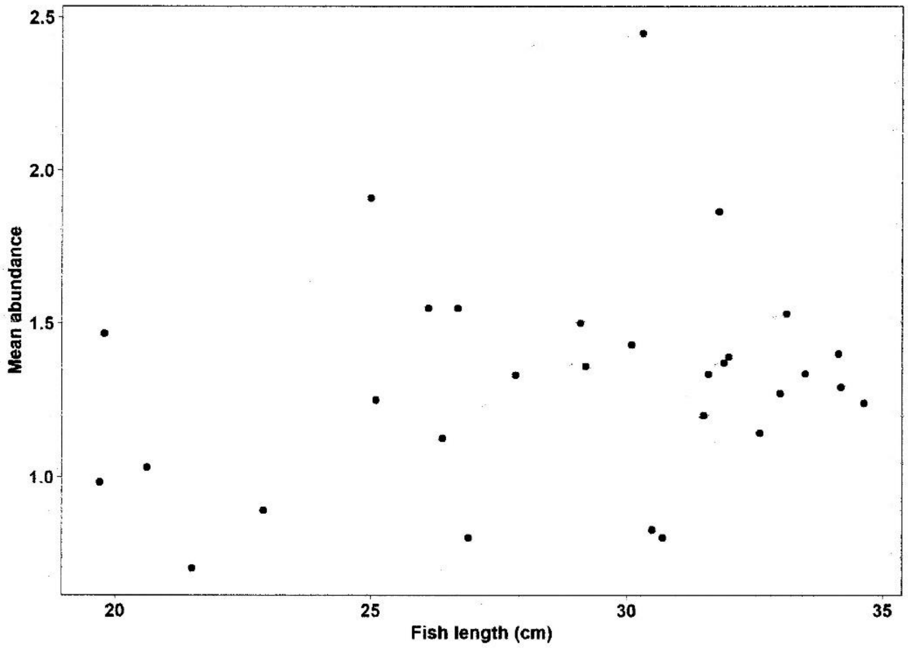


Figure 3

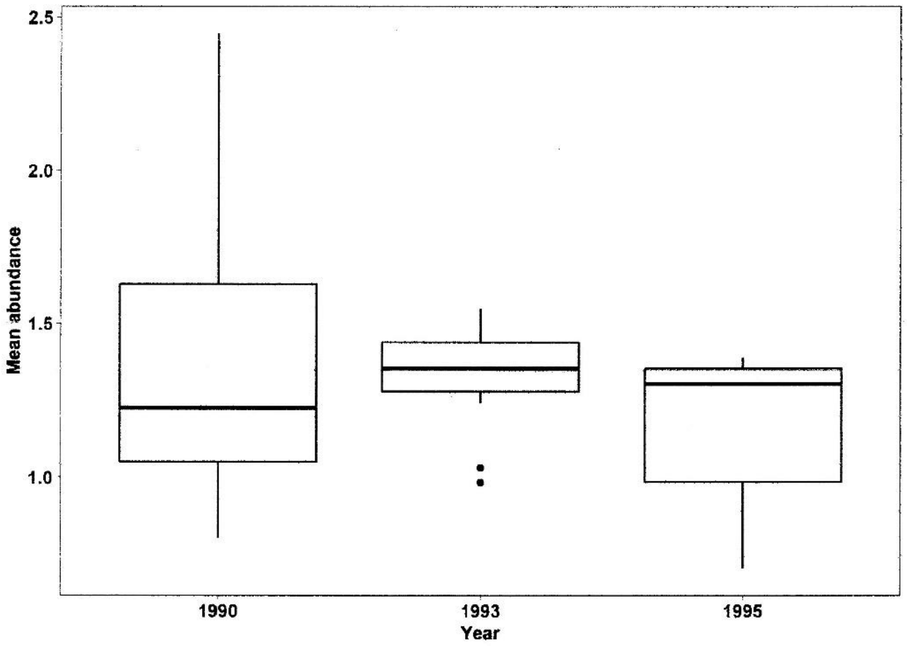
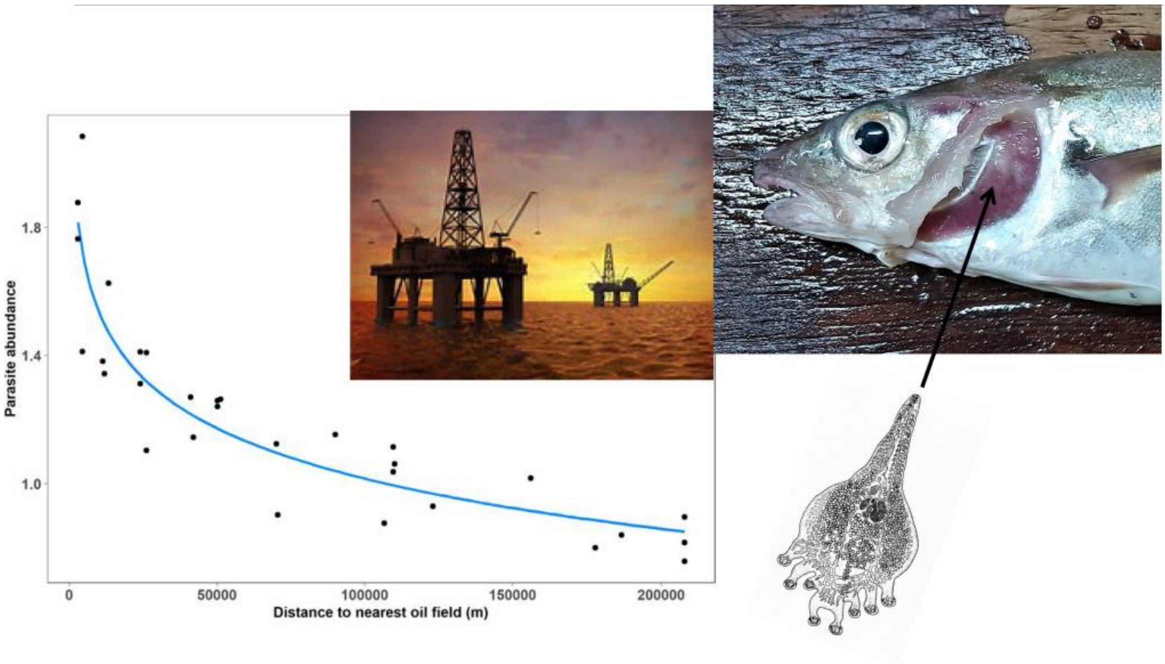


Figure 4



Graphic Abstract