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Research article

Predicting the effectiveness of oil recovery strategies in the marine polluted environment

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ABSTRACT

Many recent studies have focused their attention on the physiological stress experienced by marine organisms in measuring ecotoxicological responses. Here we suggest a new approach for investigating the effects of an anthropogenic pollutant on Life-History (LH) traits of marine organisms, to provide stakeholders and policy makers an effective tool to evaluate the best environmental recovery strategies and plans. A Dynamic Energy Budget (DEB), coupled with a biophysical model was used to predict the effects of a six-month oil spill on *Mytilus galloprovincialis'* LH traits and to test two potential recovery strategies in the central Mediterranean Sea. Oxygen consumption rates were used to check for increasing energetic maintenance costs [$\dot{p}_M$] respectively in oil-polluted system treatments (~76.2%) and polluted systems with physical (nano-bubbles ~32.6%) or chemical treatment (dispersant ~18.4%). Our model outputs highlighted a higher growth reduction of intertidal compared to subtidal populations and contextually an effect on the reproductive output and on the maturation time of this latter. The models also enabled an estimation of the timing of the disturbance affecting both the intertidal and subtidal populations' growth and reproduction. Interestingly, results led to the identification of the chemical dispersant as being the best remediation technique in contexts of oil spill contamination.

1. Introduction

The need to investigate and predict the possible effects of anthropogenic pollutants on natural and managed ecological systems is one of the most pressing challenges facing science today. Oil spills represent one among the worst risks for marine biodiversity due to high oil container traffic (REMPEC, 2008; Portopia, 2016; Zodiatis et al., 2016) and the number of oil drilling platforms often crowding semi-enclosed seas, such as the Mediterranean basin (Mangano and Sarà, 2017). The list of unexpected oil spill accidents in the last decade is long (https://en.wikipedia.org/wiki/List_of_oil_spills) and there is a pressing urgency to deepen the potential effects on marine biota on both short (days) and mid-terms (months). Thus, studies of oil spill impact and the possible quick-intervention recovery techniques using chemical-physical compounds and/or mechanisms (*e.g.* oil skimmer, boom floating, sorbent and dispersant) on the coastal biodiversity should be encouraged in basins that harbour large biodiversity and are particularly vulnerable to unexpected extreme acute pollution events (Mangano and Sarà, 2017). Once spilled, oil often reaches and accumulates on coastal intertidal habitats (De la Huz et al., 2005), the zone between the high- and low-water marks, which is recognised worldwide as crucial in providing ecosystems goods and services (Sarà et al., 2014a) but highly threatened by human activities (Barber et al., 1995; Ansari and Ingole, 2002; Orbea et al., 2006; Xia and Boufadel, 2010). Mangroves, lagoons, salt lakes, ponds, rocky shores and pools - where the worldwide marine biodiversity concentrates (Danovaro and Pusceddu, 2007) - become potential targets as already happened in the last decade (Mexico, 2010; Philippines, 2013; Bangladesh, 2014; India, 2017). As a main consequence, investigating the potential effect of oil spills on biodiversity and the degree of recovery needed could increase our understanding of how these detrimental and extreme events can be absorbed by biota. Recovery would need to include varying strategies that use chemical-physical compounds and/or mechanism such as oil skimmer, boom floating, sorbant and dispersant methods. To disentangle the effect of oil on biodiversity is likely to be difficult because of the complexity and heterogeneity of species' responses to environmental change and the choice to perform experimental studies on sentinel organisms is historically preferred by scientists (Rice et al., 1979). Since the dawn of ecological marine scientific research, marine bivalves - and more specifically mussels - have been widely used as 'sentinel' to monitor the

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wide spectrum of pollution effects on biological responses (*e.g.* 'Mussel Watch' monitoring programs). Being habitat-forming species – HFS, mussels can be easily adopted to infer on the likelihood of associated biodiversity loss (Widdows and Donkin, 1992; Widdows et al., 1995; Salazar and Salazar, 1996; Serafim et al., 2008; Sarà et al., 2013a, 2014a). Mussels can survive in the presence of both moderate trophic enriched conditions (*i.e.* suspended chlorophyll-a concentration around 1 μg l^{-1} and beyond; Sarà et al., 2011b) and high pollution levels (Halldórsson et al., 2005) buffering the human-driven biodiversity loss and recording changes in the environmental quality status of aquatic habitats at local scale (Cajaraville et al., 1996). For all these reasons, mussels have been widely used as indicators of environmental pollution (Phillips, 1976) and adopted as model organisms for physiological, genetic, toxicological and ecological studies (Smolders et al., 2003; Luedeking and Koehler, 2004; Halldórsson et al., 2007; Moore et al., 2006; Browne et al., 2008). This is also testified by the growing interest in the biological monitoring role of these sessile filter feeders, recently included in the European Marine Strategy Framework Directive (MSFD, Descriptor 9, EU 2008; Gorbi et al., 2008; Scarpato et al., 2010; Andral et al., 2011) thus recognised useful site-specific bio-indicators to meet the EU Good Environmental Status (GES). The effect of pollutants on bivalves has been frequently assessed by using the Scope for Growth (SFG) approach (Widdows and Staff, 2006; Mubiana and Blust, 2007) which allowed essentially to gain a static snapshot of the current physiological status of target organisms (Widdows et al., 1995). The success of SFG (Sobral and Widdows, 1997; Sarà et al., 2000, 2008; Widdows and Staff, 2006; Halldórsson et al., 2007; Sarà and Pusceddu, 2008; Ezgeta-Balic et al., 2011) was based on the provision of an instantaneous measure of the energy status of these key-species which was used as an indicator of the 'health' of the ecosystem (Thompson and Bayne, 1974; Widdows et al., 1995; Kearney, 2012). Nevertheless, SFG did not maximize the mechanistic power of a bioenergetic approach when assessing the bottle-necks in the energy flow from the environment to the organisms, neglecting a full translation of effects in terms of Life History (LH) traits (*e.g.* habitat body size, spawning events and Darwinian fitness; Kearney, 2012). In contrast, most recently developed bioenergetics frameworks, such as the mechanistic functional trait-based (FT) models, which rely on the Dynamic Energy Budget Theory (DEB; Kooijman, 2010; Sarà et al., 2014b), allow an easier spatially-explicit contextualisation of effects (Sarà et al., 2011a; Sarà et al., 2013a; Sarà et al., 2018a,b; Mangano et al., 2018) promising to trace new paths for future restoration strategies by predicting organismal functional traits and capturing variation across species (Pouvreau et al., 2006; Pecquerie et al., 2010; Lika et al., 2011; Sarà et al., 2011a; Kearney, 2012; Sarà et al., 2012; Sarà et al., 2013a; b; Sarà et al., 2018a,b; Mangano et al., 2018). The FT-DEB approach is based on flux of energy and mass through an organism (and not on a snapshot as in a context of the SFG approach), which is a traceable process being subject to conservation laws (Denny and Helmuth, 2009; Denny and Benedetti-Cecchi, 2012; Carrington et al., 2015). Here, an FT-DEB was spatially explicit-contextualised along the Sicilian coasts (Helmuth, 1998; Kearney et al., 2010; Sarà et al., 2011a; Sarà et al., 2012; Sarà et al., 2013a,b) in order to test the role of an acute contaminant exposure and of two recovery strategies: a commercial chemical dispersant and a nano-bubble generator (see Materials and Methods section for more details). The effects of the acute contaminant exposure and the two recovery strategies was tested on the LH traits of the blue Mediterranean mussel (*M. galloprovincialis* Lamarck, 1819), one among the most abundant filter feeders in both natural and human hard substrata (*e.g.* harbours, oil-drilling platform; Andaloro et al., 2011; Maggi et al., 2014; D'Alessandro et al., 2016; Mangano and Sarà, 2017; Mangano et al., 2017). The Sicilian waters were chosen as a target oceanographic area, which is a recognised biodiversity hotspot (Medail and Quezel, 1999) subject to high risk of accidental oil spill because it holds a central crossroad position in the Mediterranean which is the largest oil traffic route in the world (Galgani et al., 2011) and hosts the second largest oil container harbour in Europe (Augusta, Southern Sicily).

The outcomes presented and discussed are the resulting integration of an experimental and modelling study settled up to investigate the acute effect of an accidental oil spill exposure and of two possible bioremediation techniques on intertidal and subtidal mussels throughout their full life cycle. First, we compared the effects of an acute (48h) hypothetical oil spill along with that of an oil spill plus two potential recovery treatments on the mussels' energetic maintenance costs (as expressed in the DEB by $[\dot{p}_M]$ parameter and estimated as a metabolic extra-cost as measured by the oxygen consumption) and then measured the effect at individual level. Subsequently we introduced the measured effect by tweaking the $[\dot{p}_M]$ parameter in an explicit contextualised DEB model to investigate the potential implications in terms of *i)* maximum total shell length; *ii)* maximum wet weight; *iii)* reproductive outputs as expressed by the number of eggs produced; *iv)* time to reach sexual maturity; *v)* timing of disturbance.

Insights from the testing of the proposed remediation measures might inform policy makers and environmental technicians when assessing the best remediation techniques that would allow a quick recovery when a benthic population might be subjected to unexpected and acute pollution effects.

2. Materials and methods

2.1. Sampling and acclimation

Specimens of *Mytilus galloprovincialis* of commercial size (mean shell length = 65.7 ± 3.8 mm) were collected in late September 2017 from an aquaculture plant located in Lake Faro (38° 15′ 59.95″ N; 15° 38′ 19.56″ E), on the north-eastern point of Sicily (Messina, Italy). As previously described elsewhere (Cappello et al., 2011), Gas Chromatography–Flame Ionization Detection (GC-FID) analysis was used to reveal the presence of chemicals in lake water (data not shown). Mussels were collected by hand and transported within 30 min to the Mesocosm Facility of IAMC-CNR of Messina (Italy; Cappello and Yakimov, 2010). The mussels were carefully cleaned and placed in a 200 l aquarium filled with natural seawater at room temperature (18–20 °C) with a field salinity (37–38‰), and fed *ad libitum* with cultured *Isochrysis galbana*. According to common experimental procedures successfully adopted in studying the bioenergetics of bivalves (Sarà et al., 2008; Ezgeta-Balic et al., 2011), the mussels were acclimated for two weeks to reduce stress generated by manipulation and transport; following that 48 organisms were tagged with a permanent marker and transferred to mesocosms.

2.2. Experimental set-up

The mussels were housed in eight mesocosms of 120 L capacity to allow double replication (rectangular glass tanks 100 cm long, 30 cm deep, 40 cm wide), each filled with 100 L of natural seawater (Cappello et al., 2011) collected directly from the station "Mare Sicilia" (38° 11′ 43.54″ N, 15° 34′ 24.729″ E; Messina, Italy) by a direct pipeline from the sea (mean seawater temperature 20 ± 1 °C). Six mesocosms (indicated as OIL, OIL + D and OIL + B) were supplemented with 70 ml of Arabian Light Crude Oil (ENI Technology S.p.A; 900 mg l^{-1}) prepared as previously indicated elsewhere (Cappello et al., 2006, 2007). A commercial dispersant (Bioversal 0.1% vol/vol$_{OIL}$, BIOECOTECH s.r.l.) was added to mesocosms OIL + D, while mesocosms OIL + B were equipped with a commercial system for continuous nano-bubble generation (OxyDoser™ PUREair, Oxydoser USA). Two mesocosms without any addition of crude oil, dispersant and/or nano-bubble generator were used as a pristine control (CTRL). All treatments lasted 48 h.

2.3. Respiration rate

Oxygen consumption rates were determined as a proxy for stress effect and in order to determine the consequent alteration of the energetic cost of maintenance $[\dot{p}_M]$ (expressed as J cm^{-3} h^{-1}) after oil exposition. Oxygen consumption rates were measured within respirometric glass chambers (0.3 L) in a temperature-controlled water bath, filled with air-saturated filtered seawater, and stirred with a magnetic stirrer bar beneath a perforated glass plate (*e.g.* small Petri dish with holes) that supported each individual (Sarà and Pusceddu, 2008; Ezgeta-Balic et al., 2011). The decline in oxygen concentration was measured with a calibrated oxygen fibreglass sensor connected to a data logger (PiroScience Firesting O_2) capable of four sensor connections. A total of n = 48 mussels were used, acclimated as above and fed *ad libitum* until the day before the experiment. The decline of four animals for each session was continuously recorded for at least 1 h, excluding an initial period of ~10 min, characterised by a more rapid decline in oxygen caused by a disturbance of the sensor's temperature equilibration. Respiration rate (RR, μmol O_2 h^{-1}) was calculated as:

$$RR = (C_{t0} - C_{t1})x\ Vol_r x60(t_1 - t_0)^{-1}$$

according to (Sarà et al., 2008, 2013a; Ezgeta-Balic et al., 2011), where C_{t0} is the oxygen concentration at the beginning of the measurement, C_{t1} is the oxygen concentration at the end of the measurement, and Vol_r is the volume of water in the respirometric chamber.

2.4. Model description

According to the κ-rule (DEB theory; Kooijman, 2010) a fixed fraction (κ) of energy inside each organism is allocated to growth and somatic maintenance, while the remaining fraction (1 - κ) is allocated to maturity maintenance plus maturation or reproduction. If the general environmental condition deviates from common natural patterns (*i.e.* changes in temperature, food availability etc.) reproduction and growth are consequently reduced. A reduction in growth can be caused either by reduced food assimilation $[\dot{p}_A]$, enhanced maintenance costs $[\dot{p}_M]$, or enhanced growth costs $[\dot{p}_G]$. Using this approach, and through DEB parameters derived from (Sarà et al., 2011a), we simulated growth and reproduction of our model species, except for the variation in the maintenance costs $[\dot{p}_M]$ experimentally estimated through this study. The idea of quantitatively assess the effect of a stressor including it as a modification of a specific parameter was first introduced by Jager et al. (2016) with the so-called stress factor "s" applied to assimilation, maintenance and costs of growth. Here, oxygen consumption rates were used to derive the quantitative percentage effect of a stressor by comparing metabolism of control and stressed organisms, and then by summing/subtracting this value to the $[\dot{p}_M]$ parameter of *M. galloprovincialis* (Sarà et al., 2011a). To run DEB simulations local thermal series of selected sites were used, obtaining a first model with environmental conditions. A second model was run with the $[\dot{p}_M]$ calculated from the oxygen consumption rate measurements on *M. galloprovincialis* specimens.

2.5. Water temperature data and chlorophyll-a

The main forcing driver of shellfish LH inside DEB models is represented by mean seawater temperature (Pouvreau et al., 2006; Kearney et al., 2010; Kooijman, 2010; Sarà et al., 2011a). Thus, to run DEB simulations we used both intertidal and subtidal conditions (body temperature was expressed by the mean seawater temperature; Montalto et al., 2014) with four-years hourly data (January 2006–December 2009) of seawater temperature measured about 1 m below the surface at the closest meteo-oceanographic station held in 4 sites: Catania (LAT 37° 29′ 53.09″ N; LONG 15° 05′ 37.77″ E), Lampedusa (Pelagie Islands, Agrigento: LAT 35° 29′ 59.38″ N; LONG 12° 36′ 15.98″ E), Palermo (LAT 38° 07′ 17.08″ N; LONG 13° 22′ 16.79″ E) and Porto Empedocle (Agrigento; LAT 37° 17′ 08.72″ N; LONG 13° 31′ 36.74″ E). Data are available online from the Italian Institute of Environmental Research (ISPRA) web page (http://www.mareografico.it/). These sites were chosen in order to predict the effects of an oil spill around Sicilian coasts. The period of 4 years was chosen because it is consistent with the normal life span of most shellfish (Sarà et al., 2012, 2013b). Chlorophyll-a (CHL-a; μg L^{-1}), usually derived from satellite imageries, is widely adopted inside DEB models as a reliable food quantifier for suspension feeders (Kearney et al., 2010; Sarà et al., 2011a, 2012; 2013b, 2014b; 2018a,b; Mangano et al., 2018). Although the availability of satellite CHL-a data (EMIS website, http://emis.jrc.ec.europa.eu/) for the considered 4-year thermal dataset, our simulations were run with an average amount of food, imposing a value of 0.5 μg L^{-1} CHL-a to all models. While this condition might appear unrealistic (Miller et al., 2011), for the purpose of this study it will allow the exclusion of the effect of environmental variability of food conditions (CHL-a).

2.6. Effects on Life-History traits of bivalves

We answered to the following questions: 1) *What is the effect of oil or of the two tested remediation techniques (dispersant and nano-bubbles) on the LH-traits of bivalves?* and 2) *Will it affect both intertidal and subtidal populations in the same way?* Thus we ran models using the experimentally-derived $[\dot{p}_M]$. Outputs of simulations were (Sarà et al., 2014b): the maximum theoretical total length of shellfish (TL), the maximum total weight (TW), the total number of eggs (TRO) produced during a life-span of 4 years, and the time needed to reach gonadic maturity (TM) for each treatment. The average of these outputs across four different sites intends to answer our first question.

2.7. Timing effect

One of the less investigated aspects across the current literature is the timing effect of disturbance, *i.e.* the effect of disturbance at specific points during the life span of the mussels. This has recently been emphasised as a crucial aspect in investigating the role of disturbance, as the timing effect combines with frequency and intensity giving unpredictable scenarios. Thus, to determine if a particular timing (Miller et al., 2011) of the disturbance affects the LH-traits of the bivalves, we introduced into the model an oil spill event lasting 6-months by tweaking the DEB $[\dot{p}_M]$ parameter with the experimentally-derived one. In order to ascertain whether it might have different effects during the life span of mussels, we introduced the "six-month disturbance effect" once at the beginning of each year, obtaining a total of 104 models, both for intertidal (52 models) and subtidal population (52 models).

2.8. Statistical analysis

In order to test for significant differences in experimentally-derived respiration rates, a one-factor ANOVA with four levels (CTRL, OIL, OIL + D and OIL + B) was performed. When significant differences were detected, the Student-Newman-Keuls (SNK) post-hoc pair wise comparison of means was used (Underwood, 1997). Cochran's test was used prior to ANOVA to test the assumption of homogeneity of variance (Underwood, 1997).

3. Results

3.1. Respiration rate

Our results revealed significantly higher oxygen consumption rates from *M. galloprovincialis* specimens exposed to crude oil (OIL) (Table 1; ANOVA, $p < 0.01$). The SNK Test revealed significant differences between the OIL system and the control experiment (CTRL; no oil, no dispersant and no nano-bubbles) and in particular between OIL and OIL + D and between OIL and OIL + B. In contrast, no significant differences resulted between CTRL and OIL + D or CTRL and OIL + B. The rate of oxygen consumption measured was respectively ~82% higher (respect

Table 1
ANOVA table of results: respiration rate (RR) of *Mytilus galloprovincialis* (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant).

Source	df	RR		
		MS	F	P
Treatment (Tr)	3	45.43	7.58	***
Residuals	28	5.99		
Cochran's C				ns

to CTRL) for the OIL, ~16% higher for the OIL+D and ~31% for OIL+B treatment (Fig. 1a). The measured rates caused an increase in the [$\dot{p}_M$] of ~76% for the OIL, ~18% for the OIL+D and ~33% for OIL+B treatment (Fig. 1b).

3.2. Average effect on LH-traits

Outputs from model simulations with site-specific (Palermo, Catania, Porto Empedocle, Lampedusa) thermal series demonstrated a strong effect on the LH traits of intertidal and subtidal specimens of *M. galloprovincialis* (Table 2) compared to CTRL due to the hypothetical oil spill with a six-month duration.

3.3. Effects on intertidal populations

Our simulations with the presence of crude oil (OIL) predicted an average reduction of ~3.8% in the total length (TL), and of ~10.5% in the total weight (TW) within the four sites. The treatment carried out with addition of dispersant (OIL+D) predicted a reduction of ~1% in the total length (TL), and of ~3% in the total weight (TW). The OIL+B treatment (presence of nano-bubble generator) predicted a ~2% reduction in the total length (TL) and a ~5.2% reduction in the total weight (TW). Outputs from simulation predicted a ~4% increase in the maturation time for the OIL treatment, followed by a ~1.5% for the OIL+B and 0.8 for the OIL+D treatment increase respectively (Table 2; Fig. 2). No reproductive events occurred in our intertidal simulation in the four sites (Catania, Lampedusa, Palermo, and Porto Empedocle). For full results see Supplementary Information Table S1.

Table 2
Average mean effect (%) of a contaminant (OIL) and of two different oil recovery strategies (dispersant and nanobubbles, OIL+D and OIL+B respectively) on LH-traits of intertidal and subtidal individuals of *M. galloprovincialis* following a six-month spillover in the central Mediterranean Sea. (Treat = Treatment, TL = Total Length, WW = Wet Weight, TRO = Total Reproductive Output, TM = Time to Maturity).

Population	Treat	TL	WW	TRO	TM
Intertidal	OIL	−3.83	−10.47	–	3.76
	OIL + D	−1.06	−2.99	–	0.81
	OIL + B	−1.86	−5.16	–	1.48
Subtidal	OIL	−1.50	−4.39	−6.49	7.18
	OIL + D	−1.11	−3.27	−4.20	1.46
	OIL + B	−1.29	−3.80	−5.35	2.63

3.4. Effects on subtidal populations

Simulations with presence of crude oil (OIL) predicted an average reduction of 1.5% in the total length (TL), and of ~4.4% in the total weight (TW) within the four sites. The OIL+D treatment predicted a 1.1% reduction in the total length (TL), and a ~3.3% reduction in the total weight (TW). The OIL+B treatment predicted a ~1.3% reduction in the total length (TL), and a ~4% reduction in the total weight (TW). Outputs from simulations predicted a ~7% increase in the maturation time for the OIL treatment, followed by a ~2.6% for the OIL+B and 1.5% for the OIL+D treatment increase respectively. The OIL treatment also predicted a 6.5% reduction in the number of eggs produced (total reproductive output, TRO), while the OIL+D and the OIL+B treatments predicted respectively a 4.2% and a ~5.4% reduction (Table 2; Fig. 3). For full results see Supplementary Information Table S2.

3.5. Timing effect of disturbance

Outputs from model simulations with a precise timing of the oil spill disturbance demonstrated a differing pattern of effect between intertidal and subtidal populations. Intertidal simulations showed that the oil addition (OIL mesocosms) determined an increasing negative effect on the total length (TL) from ~0.9% in the first year to ~6.4% in the fourth year, coupled with an increasing negative effect on the wet

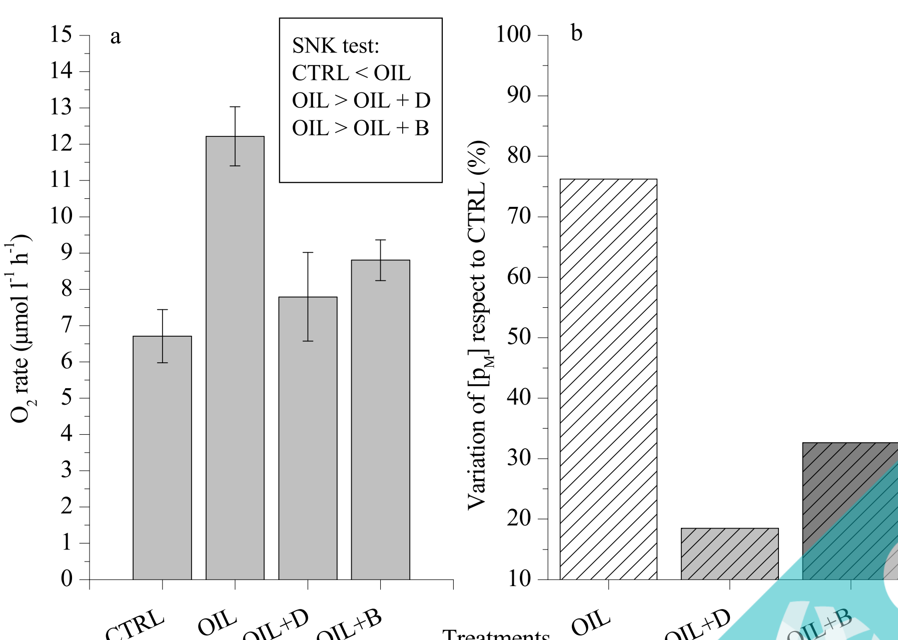

Fig. 1. (a) Oxygen consumption rates (RR) of *Mytilus galloprovincialis* under different experimental conditions; **(b)** Effect of oil addition and recovery treatments on the energetic cost of maintenance [$\dot{p}_M$], expressed in percentage in respect to control.

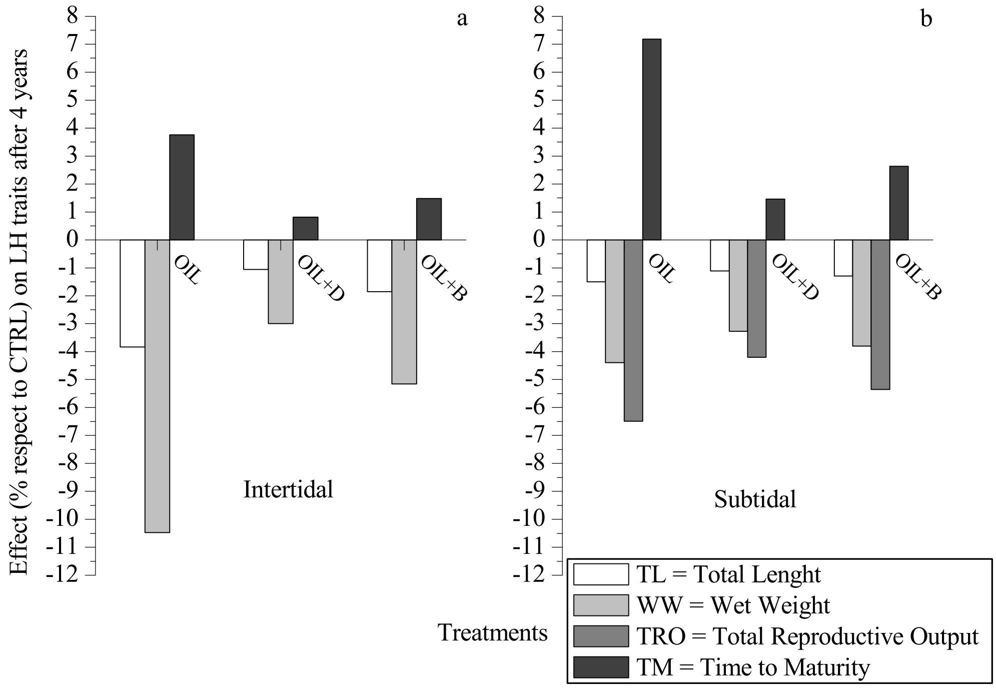

Fig. 2. Average effects on intertidal and subtidal populations from DEB simulations expressed as percentage variation of LH-Traits such as Total Length (TL), Wet Weight (WW), Total Reproductive Output (TRO) and Time to Maturity (TM) in respect to CTRL.

weight (WW) from ∼2.8% in the first year to ∼17% in the fourth year. The OIL + D treatment predicted an increasing reduction of the total length (TL) from 0.2% in the first year to 2% in the last year, coupled with an increasing reduction of the WW from 0.7% in the first year to 5.4% in the fourth year. The OIL + B treatment predicted an increasing reduction of the total length (TL) from 0.4% in the first year to ∼3.5% in the last year, coupled with an increasing reduction of the WW from ∼1.2% in the first year to ∼9.3% in the fourth year. No reproductive events occurred in our intertidal simulations but predictions reported the highest effect on the maturation time in correspondence with the second year, with an increase of 10%, 2.2% and 4.1% respectively with addition of crude oil (OIL) and two treatments (OIL + D, OIL + B) (Table 3; Fig. 4). Subtidal simulations showed that the crude oil addition (OIL) predicted an increasing negative effect on the total length

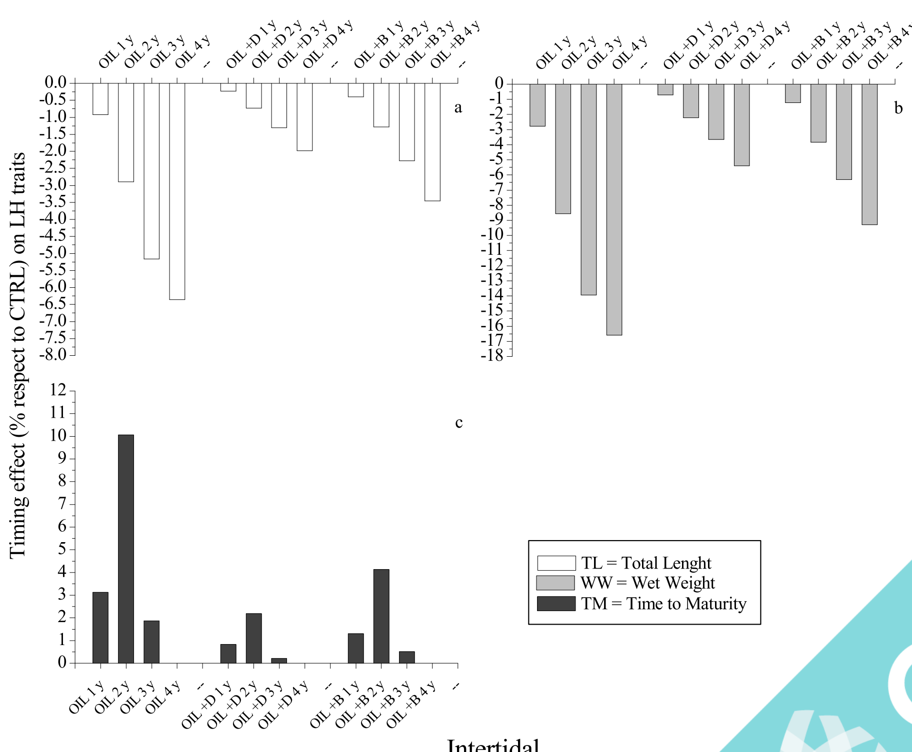

Fig. 3. Timing effect on LH-Traits of intertidal populations from DEB simulations expressed as percentage variation of **(a)** Total Length (TL), **(b)** Wet Weight (WW) and **(c)** Time to Maturity (TM) in respect to CTRL.

Table 3
Timing effect (%) of a contaminant (OIL) and of two different oil recovery strategies (dispersant and nanobubbles, OIL + D and OIL + B respectively) on LH-traits of intertidal and subtidal individuals of *M. galloprovincialis* following a six-month spillover in the central Mediterranean Sea. (Treat = Treatment, TL = Total Length, WW = Wet Weight, TRO = Total Reproductive Output, TM = Time to Maturity).

Population	Treat	TL	WW	TM	Population	TL	WW	TRO	TM
Intertidal	OIL 1 y	−0.92	−2.77	3.13	Subtidal	−0.66	−1.96	−5.9	28.71
	OIL 2 y	−2.89	−8.57	10.06		−1.89	−5.55	−12.01	0
	OIL 3 y	−5.16	−13.93	1.87		−1.75	−5.14	−8.23	0
	OIL 4 y	−6.36	−16.6	0		−1.69	−4.92	0.18	0
	OIL + D 1 y	−0.23	−0.7	0.83		−0.18	−0.53	−1.65	5.84
	OIL + D 2 y	−0.73	−2.21	2.19		−0.83	−2.47	−5.64	0
	OIL + D 3 y	−1.31	−3.64	0.21		−1.75	−5.14	−8.45	0
	OIL + D 4 y	−1.98	−5.4	0		−1.69	−4.94	−1.06	0
	OIL + B 1 y	−0.4	−1.22	1.3		−0.3	−0.91	−2.8	10.53
	OIL + B 2 y	−1.28	−3.84	4.13		−1.43	−4.22	−9.45	0
	OIL + B 3 y	−2.28	−6.3	0.51		−1.75	−5.14	−8.39	0
	OIL + B 4 y	−3.46	−9.29	0		−1.69	−4.94	−0.75	0

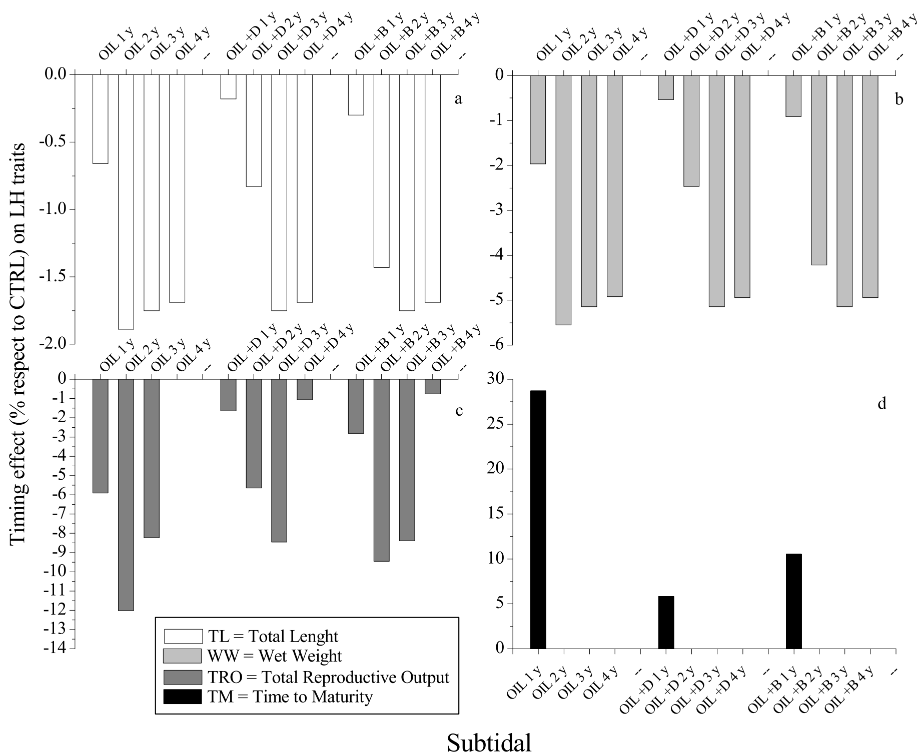

Fig. 4. Timing effect on subtidal populations from DEB simulations expressed as percentage variation of **(a)** Total Length (TL), **(b)** Wet Weight (WW), **(c)** Total Reproductive Output (TRO) and **(d)** Time to Maturity (TM) in respect to CTRL.

(TL) from ∼0.7% in the first year to ∼1.7% in the fourth year, coupled with an increasing negative effect on the WW from ∼2% in the first year to ∼5% in the fourth year. The addition of dispersant (OIL + D treatment) predicted an increasing reduction of the total length (TL) from 0.2% in the first year to 1.7% in the last year, coupled with an increasing reduction of the WW from 0.5% in the first year to ∼5% in the fourth year. The OIL + B treatment predicted an increasing reduction of the total length (TL) from 0.3% in the first year to ∼1.7% in the last year, coupled with an increasing reduction of the WW from 0.9% in the first year to ∼5% in the fourth year. In the system with only crude oil present (OIL) the total reproductive output (TRO) resulted between the second (−12%) and the third year (−8.5%). The OIL + D followed the same decreasing pattern (respectively −5.6 and −8.5%) as well as the OIL + B treatment (−9.4 and −8.4% respectively). Our simulation reported the highest effect on the maturation time in correspondence with the first year, with a +28% (OIL), a +5.8% (OIL + D) and a +10.5% (OIL + B) (Table 3; Fig. 4).

4. Discussion

Integrated modelling and experimental studies based on mechanistic simulations, to predict the effects of an oil spill on *M. galloprovincialis* populations, have never been performed before in a Mediterranean context, suggesting the importance of this tool in helping the near future remediation technique move a step forward to become the most developed integrated-monitoring-studies already successfully applied (Gorbi et al., 2008; Gomiero et al., 2011a,b). Interestingly, Gomiero and co-authors have recently performed a novel algorithm aiming at drawing up indices to rank the different stages of pollutant-induced stress syndrome, helping to translate the biomarker data into an actual health risk index to suggest to environmental managers (Mussel Expert System, MES; Gomiero et al., 2015).

Here, in order to assess the impact on shellfish fecundity we introduced 4 years' worth of hourly data of the seawater, recorded in four sites, with an average value of food, in order to exclude the interference derived from the natural resource variability. No reproductive events came out of most of our intertidal simulations, probably due to food limitations and temperature threshold. Although *M. galloprovincialis* is an autochthonous Mediterranean species, it is more frequent throughout the northern coasts of the Basin (Northern Tyrrhenian, Adriatic), while in the southern waters, it is represented by a patchy distribution except for highly eutrophic (*e.g.* Augusta Bay, Southern Sicily, Italy) or shallow waters (*e.g.* Bizerta Lake, Tunisia). A simulated six-month oil spill around Sicilian coasts through our models revealed a negative effect on growth and a stronger effect on the reproduction and the time needed to reach gonadic maturation. Model outputs revealed a twofold higher negative oil effect on the TL (−3.83 *vs.* −1.5%) and WW (−10.47 *vs.* −4.39%) of the intertidal populations in respect to subtidal ones. The latter were instead highly affected in the TRO (−6.49%) and in the TM (−7.18%). According to the DEB theory, when general environmental conditions deviate from common natural patterns, reproduction and growth can be consequently affected. Increasing maintenance costs as expressed by $[\dot{p}_M]$ is one among the most important cause driving the change in the growth and the reproductive performances of organisms. Here, we were able to demonstrate that both the remediation techniques, dispersant and nano-bubbles, showed not-significant differences in oxygen consumption rates. However, the calculation of the energetic cost of maintenance $[\dot{p}_M]$ indicates a lower increase for the OIL+D in respect to the OIL+B mesocosm (Fig. 1b) suggesting the dispersant as the best remediation technique. This was also confirmed by the model outputs, as long as they revealed a twofold higher negative OIL+B effect in TL (−1.86 *vs.* −1.06%) and WW (−5.16 *vs.* −2.99%) in respect to the OIL+D treatment for intertidal populations. Outputs also revealed some effect of the OIL+B and OIL+D treatments on subtidal populations traits, such as TL (−1.29 *vs.* −1.11%) and WW (−3.8 *vs.* −3.27%), and a more important effect on TRO (−5.35 *vs.* −4.2) and on TM (−2.63 *vs.* −1.46). The gonadal development has previously been described in literature as one of the main proxy of increasing trends of heavy metal accumulation in proximity to oil platform extraction (Gomiero et al., 2011a; Mangano and Sarà, 2017). Moreover, the estimation of the fecundity potential of intertidal and subtidal populations, which is often omitted in other ecological studies, has great importance, both for ecological aquaculture and conservation management.

Our results can be explained by the faster physical effect of dispersant with respect to the slower nano-bubbles initial contribution. The dispersant is able to break down the slick of oil into smaller droplets and facilitates their transfer throughout the water column determining a rapid dilution reducing the oil exposure of the organisms. The ability to target the thickest part of the oil slick in a timely manner, before oil weathering reduce the efficiency of the action is one of the key success factors of dispersant application in oils spill response. The other fundamental advantage is their easiness of application through a variety of methods (*e.g.* spraying dispersant from vessels and small aircraft or helicopters) in the case of oil spills. In contrast, nano-bubbles tend initially to favor the oil water accumulation in the water upper layers by contrasting the mixing effects of the water turbulence. Over a longer period, the increased amount of oxygen and organic load at the surface can stimulate biodegradation activity of hydrocarbonoclastic bacteria (Ohnari, 1997; Moriguchi and Kato, 2002). Due to the limited duration of the mesocosm experiments, we assumed that the performance of the two applied techniques, in terms of reducing the availability of the contaminant to the organisms, was higher for the dispersant than nano-bubbles according to the faster physical effect of the first on the oil concentration. However, over a longer observation period, the efficiency of the two selected approaches should be more comparable due to the increased contribution of microbial activity. Nevertheless, recent research showed that in marine open water, the application of nano-bubbles may be not easily practicable due the "turbulence effect" which facilitate the dispersion. Thus the application of nano-bubbles should be mainly focused in closed basins or in oily-wastewater treatment plants (Ohnari, 2001; Li and Tsuge, 2006).

4.1. Implications for biodiversity

Biodiversity is widely recognizes as one of the fundamental provider of ecosystem services (Loreau et al., 2001; Tilman et al., 2014) affecting ecosystem productivity, nutrient cycling, stability and resistance to perturbations (Naeem et al., 1994; Smith et al., 2006; Mazancourt et al., 2013). On the other hand, anthropogenic disturbance is one of the most powerful driver capable to shape biodiversity (Miller et al., 2011) through a driving action on individual "life histories" (Denny et al., 2009; Sarà et al., 2018a). Looking at our case study, an oil spill can act not only at individual level but hierarchically, through the effect on hundreds of mussel-beds associated species (Suchanek, 1979), alter the ecosystem functioning in terms of provided good and services (Sarà et al., 2014a,b; Mangano and Sarà, 2017). Although modelling is an extremely specific tool, it is possible to generalize the effect of an oil spill as largely dependent on species' mobility and the possibility of escaping toxicity (Loureiro et al., 2006), resulting in a higher vulnerability of sessile and aquaculture species. By tailoring this model to other model species in the future it may be possible to detect the cumulative effect of oil spill on specific sectors (*e.g.* fishery, aquaculture) and aquatic environments (*e.g.* marine, freshwater, saltmarsh). An expansion of the knowledge on the needed degree of recovery could increase our understanding of how oil spill detrimental and extreme events can be absorbed by biota. In the future, recovery would need to include integrated strategies more specific based on various remediation techniques (*i.e.* chemical-physical compounds and/or mechanism such as oil skimmer, boom floating, sorbant and dispersant methods) to adapt to species responses and preserve the associated biodiversity functioning.

5. Conclusions

Our simulations finally led also to predict the timing effect of the disturbance that demonstrated a differing pattern between intertidal and subtidal populations. From the comparison of model outputs, it has been demonstrated that the disturbance strongly affected the growth of the intertidal populations from the second to the fourth year, and maturation mostly in the first and second year. The disturbance instead acted on subtidal population with a lower intensity on growth across the period from the second to the fourth year, but with a higher intensity on maturation during the first year, affecting the reproduction mostly in the second and third year.

There are actually no data in the current literature regarding the timing of disturbance to refer to, or compare with, so this actually represent the first exercise to assess and predict the differing effects of a contaminant across the life-span of a model species through mechanistic models. Our pilot experiment, coupled with bioenergetics models predicting the growth and the potential fecundity of a model species proposes a new approach in testing, on a broad spatial and time scale, the effects of any anthropogenic pollutant, and as a potential tool that will lead stakeholders and policy makers to evaluate current, and propose future, remediation strategies to achieve the Good Environmental Status. On a small scale, this method could be easily applied even to a single drilling platform at sea, in order to investigate the potential impact on nearby coasts and to design in advance the most effective technique to be adopted in case of an environmental emergency or oil-spills during the platforms' maintenance and dismiss phases.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2018.06.094.

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