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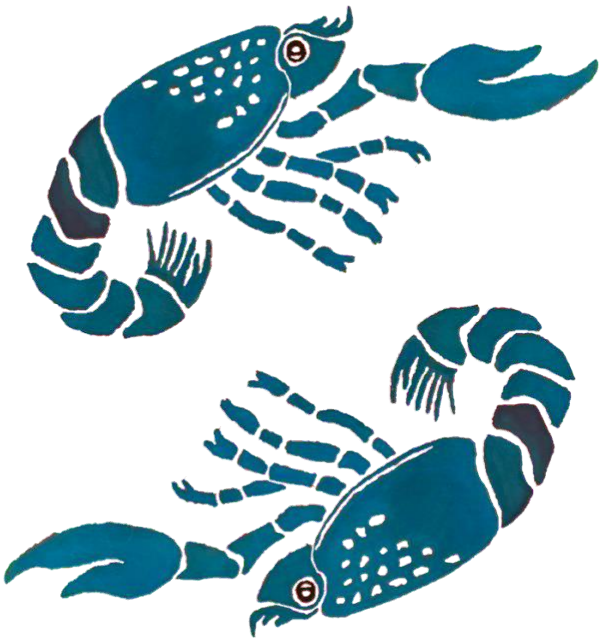
Effects of anthropogenic stressors on Helgoland's lobsters (*Homarus gammarus)*



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FREQUENTLY USED ABBREVIATIONS

AIC: Akaike information criterion

AL: Abdomen length

C: Carbon

CAT: Catalase

CL: Carapace length

GPx: Glutathione peroxidase

GST: Glutathione S-transferase

IPCC: Intergovernmental Panel on Climate Change

MDA: Malondialdehyde

N: Nitrogen

OA: Ocean acidification

OWF: Offshore windfarms

***p*CO_2:** Partial pressure of carbon dioxide

RL: Rostrum length

RMR: Routine metabolic rate

SOD: Superoxide dismutase

SSP: Socio-economic pathway

SST: Sea surface temperature

TL: Total length

YOY: Young-of-year

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THESIS SUMMARY

As meroplankton, lobsters make up a great portion of both benthic communities and planktonic fauna in the water column. Furthermore, they represent a mayor food source across the marine food web and a vital source of protein for humans. As an economically important species, lobsters are highly susceptible to anthropogenic stressors (e.g habitat destruction, over-fishing, noise pollution). Moreover, climate change may magnify the impact of human activities on lobsters' fitness. The collapse of the population of European lobster (*Homarus gammarus*) around Helgoland constitutes a good example and prompted a large-scale restocking program. Yet, the question arises if recruitment of remaining natural individuals and program released specimens could be stunted by ongoing climate change and human activities.

In my thesis I investigate the effect of several anthropogenic stressors that could potentially be affecting the route to recovery of Helgoland's lobsters. Since monitoring of lobster larvae has not been implemented on the island yet, I performed laboratory and field experiments to evaluate the potential of using light-traps on the island of Helgoland to catch lobster larvae. Developing adequate traps is useful to support research on the European lobster in the wild and conservation efforts. The light traps used white LED lights and successfully trapped lobster larvae in laboratory experiments at different densities of 100 and 10 ind/m^3 , and in the field experiment, six traps were deployed weekly throughout lobster larvae hatching season in the months of May to August. Traps were deployed in two different rocky seabed sites, that are adequate habitats for larvae, and at different depths (1, 2, 4, and 6 m). However, no lobster larvae were caught in the field. This may be due to several reasons: (1) low population numbers of lobster larvae, (2) a rapid loss of positive phototactic response of larvae to light or (3) the chosen deployment sites were not appropriate. Future research is needed to construct a specialized trap to sample Helgoland's lobster larvae and provide information on the current larval fitness and population numbers. Nevertheless, the light traps used in my thesis successfully caught a variety of small crustaceans and can be a useful tool to sample small benthic organisms for experimental purposes.

Owing to the difficulties in catching lobster larvae in the field, I used larvae from lobster-rearing facilities to study the effects of anthropogenic stress on larval development and physiology. Studies on the effects of climate change on European lobster larvae have mostly focused on the isolated effect of ocean acidification or warming. Acidification treatments were based on two shared socio-economic pathways emitted by the Intergovernmental Panel on Climate Change (IPCC) regarding the amount of atmospheric CO_2 for the end of the century. This study is the first to provide a more complete picture of the thermal limits at different levels of biological organization of lobster larvae under acidification by including a ten-level temperature gradient setup (13-24°C) The results show temperature was positively correlated with growth and energy metabolism; while, pCO_2 had a negative impact on survival and morphology. Thus, climate change could potentially stunt the European lobster restocking efforts taking place on the island.

Lastly, I investigated the impact noise pollution may have on juvenile lobster's behavior and substrate choice. Around the island of Helgoland there are numerous offshore windfarms (OWF) which are a source of constant low-frequency noise. As OWF foundations also have the potential to act as a hard substrate habitat for lobsters, I assessed the separate and joint effect of predator presence and a constant low-frequency noise on young-of-year European lobsters' behavior and substrate choice. The results suggest that the single and joint effect of added noise and predator presence influenced their initial substrate choice. Furthermore, young-of-year lobsters under the added noise and predator presence treatment spent more time outside their substrate (shelter), exploring, and less time hiding. The latter is ecologically detrimental as animals may expose themselves more to predator in natural conditions. These behavioral responses raise concerns about the effects constant low-frequency noise may have on young-of-year and adult European lobster living among artificial reef structures such as foundations of operational offshore wind farm naturally, or in the frame of restocking, multi-use and temporary no-take zones programs.

This thesis documents a field sampling trial with light traps that, while failing its main aim for lobster larvae, it could be followed up to obtain more abundance and biomass data on epibenthic specimens. Nevertheless, with specimens obtained from rearing-facilities, the thesis provides a comprehensive and contemporary picture of the effects of anthropogenic

stressors on the early-life stages of the European lobster larvae, physiologically and behaviorally. Ocean warming and acidification can lead to an increase in mortality and lower rostrum size in lobster larvae, while noise pollution can disturb the behavior of juvenile lobsters, thus affecting their attention and decision-making processes. Helgoland's lobsters are a vulnerable population that will continue to be affected by offshore human activity and climate change, therefore restocking programs, scientific support and fisheries management should also be a permanent action.

ZUSAMMENFASSUNG

Als Meroplankton machen Hummer einen großen Teil sowohl der benthischen Gemeinschaften als auch der planktischen Fauna in der Wassersäule aus. Darüber hinaus stellen sie eine wichtige Nahrungsquelle im marinen Nahrungsnetz und eine wesentliche Proteinquelle für den Menschen dar. Als wirtschaftlich wichtige Art sind Hummer sehr anfällig für anthropogene Stressoren (z. B. Zerstörung von Lebensräumen, Überfischung, Lärmbelästigung). Darüber hinaus kann der Klimawandel die Auswirkungen menschlicher Aktivitäten auf die Fitness von Hummern noch verstärken. Der Zusammenbruch der Population des Europäischen Hummers (Homarus gammarus) um Helgoland ist ein gutes Beispiel und war Anlass für ein groß angelegtes Wiederaufstockungsprogramm. Es stellt sich jedoch die Frage, ob die Rekrutierung der verbleibenden natürlichen Individuen und der im Rahmen des Programms freigelassenen Exemplare durch den Klimawandel und menschliche Aktivitäten beeinträchtigt werden könnte.

In meiner Dissertation untersuche ich die Auswirkungen verschiedener anthropogener Stressoren, die den Weg zur Erholung des Helgoländer Hummers möglicherweise beeinträchtigen könnten. Da die Überwachung von Hummerlarven auf der Insel noch nicht eingeführt wurde, führte ich Labor- und Feldexperimente durch, um das Potenzial des Einsatzes von Lichtfallen auf der Insel Helgoland zum Fang von Hummerlarven zu bewerten. Die Entwicklung geeigneter Fallen ist nützlich, um die Erforschung des Europäischen Hummers in der freien Natur und die Bemühungen um seine Erhaltung zu unterstützen. Die Lichtfallen wurden mit weißen LED-Leuchten bestückt und fingen in

Laborexperimenten erfolgreich Hummerlarven in unterschiedlichen Dichten von 100 und 10 ind/m^3. Im Feldexperiment wurden sechs Fallen wöchentlich während der Schlupfzeit der Hummerlarven in den Monaten Mai bis August aufgestellt. Die Fallen wurden an zwei verschiedenen Stellen des felsigen Meeresbodens, die geeignete Lebensräume für die Larven darstellen und in unterschiedlichen Tiefen (1, 2, 4 und 6 m) aufgestellt. Vor Ort wurden jedoch keine Hummerlarven gefangen. Dies kann mehrere Gründe haben: (1) geringe Populationszahlen von Hummerlarven, (2) ein schneller Verlust der positiven phototaktischen Reaktion der Larven auf Licht oder (3) die gewählten Einsatzorte waren nicht geeignet. Zukünftige Forschungsarbeiten sind erforderlich, um eine spezielle Falle für die Probenahme der Helgoländer Hummerlarven zu konstruieren und Informationen über die aktuelle Fitness der Larven und die Populationszahlen zu erhalten. Nichtsdestotrotz haben die in meiner Arbeit verwendeten Lichtfallen erfolgreich eine Vielzahl kleiner Krebstiere gefangen und können ein nützliches Instrument sein, um kleine benthische Organismen für experimentelle Zwecke zu beproben.

Da es schwierig ist, Hummerlarven im Freiland zu fangen, habe ich Larven aus Hummeraufzuchtanlagen verwendet, um die Auswirkungen von anthropogenem Stress auf die Entwicklung und Physiologie der Larven zu untersuchen. Studien über die Auswirkungen des Klimawandels auf europäische Hummerlarven haben sich meist auf die isolierten Auswirkungen der Versauerung oder Erwärmung der Ozeane konzentriert. Bei der Untersuchung der Versauerung wurden zwei gemeinsame sozioökonomische Pfade zugrunde gelegt, die das Intergovernmental Panel on Climate Change (IPCC) für die Menge an atmosphärischem CO_2 am Ende des Jahrhunderts vorgibt. Diese Studie ist die erste, die ein vollständigeres Bild der thermischen Grenzen auf verschiedenen Ebenen der biologischen Organisation von Hummerlarven unter einer Versauerung liefert, indem sie einen zehnstufigen Temperaturgradienten (13-24°C) einbezieht. Die Ergebnisse zeigen, dass die Temperatur positiv mit dem Wachstum und dem Energiestoffwechsel korreliert, während der pCO_2-Wert einen negativen Einfluss auf das Überleben und die Morphologie hat. Somit könnte der Klimawandel die Bemühungen zur Wiederaufstockung der europäischen Hummerbestände auf der Insel möglicherweise zunichte machen.

Schließlich untersuchte ich, wie sich die Lärmbelastung auf das Verhalten und die Substratwahl der Junghummer auswirkt. Rund um die Insel Helgoland gibt es zahlreiche Offshore-Windparks (OWF), die eine Quelle ständigen niederfrequenten Lärms darstellen. Da OWF-Fundamente auch das Potenzial haben, als Hartsubstrat-Habitat für Hummer zu fungieren, habe ich die getrennten und gemeinsamen Auswirkungen der Anwesenheit von Raubtieren und eines konstanten tieffrequenten Lärms auf das Verhalten und die Substratwahl von jungen europäischen Hummern untersucht. Die Ergebnisse deuten darauf hin, dass die einzelnen und gemeinsamen Auswirkungen von zusätzlichem Lärm und der Anwesenheit von Raubtieren ihre anfängliche Substratwahl beeinflussten. Darüber hinaus verbrachten junge Hummer unter der Behandlung mit zusätzlichem Lärm und der Anwesenheit von Raubtieren mehr Zeit außerhalb ihres Substrats (Unterschlupf), um es zu erkunden und weniger Zeit, um sich zu verstecken. Letzteres ist ökologisch nachteilig, da sich die Tiere unter natürlichen Bedingungen mehr Raubtieren aussetzen könnten. Diese Verhaltensweisen geben Anlass zur Besorgnis über die Auswirkungen, die konstanter tieffrequenter Lärm auf junge und ausgewachsene Hummer haben kann, die zwischen künstlichen Riffstrukturen, wie z. B. Fundamenten von Offshore-Windparks, oder im Rahmen von Programmen zur Wiederaufstockung von Beständen, zur Mehrfachnutzung und zur Einrichtung vorübergehender Nichtentnahmezonen leben.

Diese Arbeit dokumentiert einen Feldversuch mit Lichtfallen, der zwar sein Hauptziel für Hummerlarven nicht erfüllte, aber weiterverfolgt werden könnte, um mehr Abundanz- und Biomassedaten über epibenthische Exemplare zu erhalten. Darüber hinaus liefert die Arbeit mit Proben aus einer Aufzuchtanlage ein umfassendes und aktuelles Bild der Auswirkungen anthropogener Stressoren auf die frühen Lebensstadien der europäischen Hummerlarven in physiologischer und verhaltensmäßiger Hinsicht. Die Erwärmung und Versauerung der Ozeane kann zu einer erhöhten Sterblichkeit und einer geringeren Größe der Hummerlarven führen, während die Lärmbelastung das Verhalten der Junghummer stören und damit ihre Aufmerksamkeit und Entscheidungsprozesse beeinträchtigen kann. Die Helgoländer Hummer sind eine gefährdete Population, die auch weiterhin durch menschliche Aktivitäten vor der Küste und den Klimawandel beeinträchtigt werden wird, weshalb Wiederaufstockungs-programme, wissenschaftliche Unterstützung und Fischereimanagement auch eine dauerhafte Maßnahme sein sollten.

CHAPTER 1. GENERAL INTRODUCTION

Crustaceans are indispensable to the earth's biodiversity; since they represent the largest group of invertebrate animals, which includes crabs, lobsters, shrimps, krill, copepods, amphipods and more sessile organisms like barnacles. From microscopic copepods to giant spider crabs spanning more than 3 m, crustaceans are key in maintaining healthy and balanced ecosystems. Small crustaceans make a substantial portion of the primary consumers in the marine food web and as filter feeders, can recycle nutrients. Larger crustaceans are top predators and regulators in benthic ecosystems and additionally act as food source for large fish and aquatic mammals. Crustaceans' main characteristics are their exoskeleton, two pairs of antennae, a pair of mandibles, two compound eyes (often on stalks) and segmented bodies with appendages on each segment. The number of appendages is key in classifying some members of this vast taxon into the Decapoda (literally "ten-footed) order. Most decapods are found in aquatic environments spending a major part of their life cycle on the ocean floors and are exploited by coastal and offshore fisheries, especially clawed lobsters.

Lobster species sustain some of the most profitable fisheries in tropical, subtropical and temperate waters worldwide. Due to their socio-economic importance, their reproduction and life cycle has been widely studied and documented. Additionally, because of their size, and perceived exclusivity they have become regional flagship species and sparked the interest of the scientific community in efforts to enhance the sustainability of the lobster industry. Among the diversity of lobsters, the genus *Homarus* includes two widely fished species, the American lobster (*Homarus americanus)* and the European lobster (*Homarus gammarus*). As their name implies, the American lobster can be found along the American continent, from Labrador to the United States of America (USA) mid-Atlantic, while the European lobsters' habitat extends along the west coast of Europe, from northern

Norway, to the Iberian Peninsula and further south to the Azores and Morocco; they are also present in most of the Mediterranean Sea (Holthuis, 1991). Despite the abundance of research on *Homarus* lobsters, most studies have been conducted in populations of USA, Canada, United Kingdom (UK) and Norway. However, there exist clusters of lobsters in more remote areas, such as a population of European lobsters off the island Helgoland (54°11.3'N, 7°54.0'E, German Bight, North Sea). Our understanding of the impact increasing offshore activities and climate change will have on this particular lobster population remains incomplete.

In the following introductory sections, an overview is given on the life cycle of European lobsters as well as the anthropogenic stressors they are currently facing. Special attention is given to the description of this German Bight population, its fisheries and the local lobster conservation efforts.

1.1 Helgoland's lobsters

European lobsters' preferred habitats are sedimentary and rocky bottoms with crevices and boulders. In the southern-eastern North Sea, the seafloor is sand and mud-dominated, and this is generally assumed as the limiting factor for lobsters to settle in the German Bight. European lobsters are rarely encountered in the German Bight, except in a few isolated wrecks and on the rocky island of Helgoland, which offers an ideal habitat (Krone and Schröder, 2011). The island of Helgoland is surrounded by and intertidal and subtidal hard bottom covering about 35 km^2 with a maximum depth of 24 m (Hickel, 1972). Helgoland's lobsters are intertwined with the island's fishing culture and were a major part of the island's income from the 19th century to the 1930s (Ehrenbaum, 1894), when lobsters catches amounted up to 80,000 animals annually (Klimpel, 1965). However, since the 1950s, the catch has declined drastically reaching a minimum of ~100 animals annually in the 1990s (Goemann, 1990). A combination of overfishing, pollution and extensive habitat destruction by the bombing of the island during and after the Second World War, are all pointed out to

explain the collapse in fisheries yields and population densities (Anger and Harms, 1994; Franke and Gutow, 2004).

Legislative regulations and conservation efforts may have helped prevent the complete disappearance of the local stock. These regulations include the agreement on a minimum landing size of 85 mm carapace length, a ban on fishing berried females and a closed season between the months of July-August (Schmalenbach et al., 2011). In the years 2006-2009 the catch per unit effort (CPUE) increased significantly based on data provided by lobster fishermen (from 0.01 to 0.02 of lobster per pot lift) (Schmalenbach et al., 2011). However, these regulations are not sufficient to ensure the recovery of the population, and within the German waters the European lobsters are considered a highly endangered species (Rachor et al., 1998). Since the Helgoland lobster population is isolated in the southern North Sea, it is improbable the stock will recover through resettlement of lobsters from neighboring areas in UK and Denmark. Therefore, beside fishing regulations, the Alfred-Wegener-Institut (AWI) Helmholtz-Zentrum für Polar-und Meeresforschung-Biologische Anstalt Helgoland with support of the Ministry of Fisheries and Agriculture of the State of Schleswig-Holstein in Germany initiated a program between 1999 and 2009 to investigate whether lobster restocking was possible (Schmalenbach et al., 2011). Through this initiative, a total of over 14,000 juvenile lobsters were released between 2000 and 2017 around Helgoland. Nowadays, restocking has taken off in earnest through the lobster conservation company Reefauna, with up to 6000 juvenile lobsters released annually. Yet the population numbers remain low due to the very small catch of 200-300 lobster per year (Schmalenbach, 2016, 2017) which still remains below the critical threshold necessary for a recovery at a large scale. Furthermore, even though lobster monitoring is done twice per year around the island of Helgoland by AWI, little is known on the whole composition and dynamics of the Helgoland lobster population. Only adult lobsters are caught or seen around the island, however early life-stages like larvae and juveniles have seldomly been caught. Therefore, the question remains if recruitment of remaining natural individuals and program-released specimens is currently successful. Lobster larvae are rarely found in AWI long-term plankton sampling (Helgoland Roads), possibly because the CalCoFI plankton nets are not suitable for lobster larvae behavior and a more specific trap should be deployed. Zooplankton can avoid or escape nets to an extent, especially stronger swimmers like crustaceans larvae (Fleminger and Clutter, 1965; Brinton,

1967; Guest et al., 2003). Therefore, stationary traps are better suited for the capture of crustacean larvae. Besides net avoidance, the time of the day in which plankton nets are deployed will strongly influence the “catchability” of organisms. Lobster larvae are positively phototactic and can be lured with light during nighttime. Thus, different designs of light traps ought to be tested to successfully catch lobster larvae and have a better estimation of larval densities on Helgoland.

Due to the challenges to find them in the wild, studies on these life-stages are limited to aquarium experiments using hatchery reared larvae and juveniles. Several scientists have studied European lobsters at the Biologische Anstalt Helgoland. The most recent studies have focused on the effects of food quality on larvae (Schoo et al., 2012, 2014), larval and juvenile behavior Schmalenbach and Buchholz, 2013) and the physiological impacts of changes in abiotic marine parameters like temperature and salinity (Torres et al., 2021). As well as aquaculture studies on improving the rearing conditions for juvenile lobsters (Schmalenbach et al., 2009). Yet more studies are needed on additional anthropogenic stressors lobsters are facing (i.e. ocean acidification and noise pollution), which may be restricting the recovery of Helgoland’s lobster population.

1.2 European lobster life cycle

Lobsters are representative of benthic species with complex life cycles, similarly to many marine invertebrates (e.g echinoderms, mollusks, crustaceans). The majority of crustaceans undergo a pelagic larval phase, and then a juvenile to adult benthic phase. Larvae are characterized by the absence of adult features and thus differ from conspecific juveniles and adults based on morphological, ecological, behavioral and physiological traits (Martin et al., 2014; Anger et al., 2020).

The European lobster life cycle starts as an egg attached for ~9-11 months to the abdomen of an adult female (Mehrtens, 2011). After hatching, larvae molt successively through three pelagic larval stages (Stage I-III) and a benthic postlarvae stage (stage IV) (Charmantier et al., 1991a) (Figure 1).

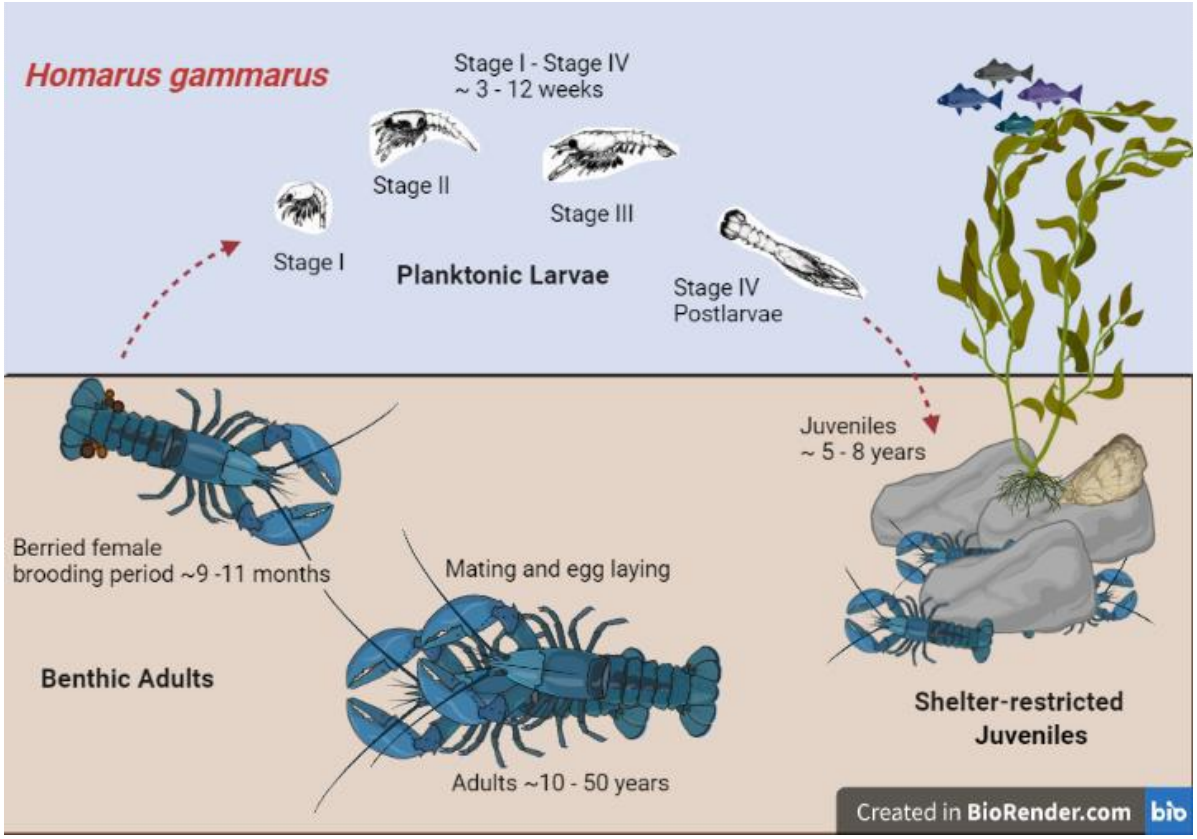

Figure 1. European lobster life cycle. Created with BioRender.com and stage I – IV larvae modified from nationallobsterhatchery.co.uk

The planktonic larval stages last between 3-10 weeks during which natural mortality awaits larvae mostly from predation and sensitivity to suboptimal environmental conditions in the water column (MacKenzie, 1988). The transition between the last larval pelagic stage (III) to the benthic post-larval stage (IV) is also associated with high mortality and has been described as a population bottleneck within the lobster lifecycle (MacKenzie, 1988; Marshall and Morgan, 2011). When lobsters reach the juvenile stage, this marks the complete transition to a benthic life style (Charmantier et al., 1991a). Juvenile stage is characterized by a strong dependency on finding a suitable shelter for protection. Juvenile lobsters are nocturnally active and spent most of the daytime hiding in complex shelters made of rocks, reefs and seaweed (Able et al., 1988; Lawton and Lavalli, 1995; Mehrtens et al., 2005b).

After five to eight years, European lobsters reach sexual maturity and look for suitable partners to mate with in late summer (Mehrtens, 2011). Mating usually occurs between a freshly molted female and a hard-shelled male (van der Meeren et al., 2008; Skog, 2009).

After fertilization, the adult female carries the eggs; while they change color from black to orange as they develop. Finally, hatching occurs the following year between May and August, with a peak in numbers in July (Mehrtens, 2011).

1.3 Anthropogenic stressors

Global climate change is one of the most far-reaching human transformations on the Earth, and the greatest threat to our ecosystems and livelihood (Rosenzweig et al., 2008). The effects of climate changes are especially evident in the impacts on the biodiversity, structure and functions of marine ecosystems. The exponential increase of CO_2 emissions since the industrial age is causing the oceans to become warmer and more acidic. According to the Intergovernmental Panel on Climate Change (IPCC) if mitigation efforts to decrease CO_2 emissions are not taken earnestly; sea surface temperature (SST) could increase by 2.58°C and seawater surface pH could drop to 7.7 by the year 2100, as predicted by the low mitigation scenario SSP5-8.5. However, if the high mitigation scenario SSP1-2.6 is implemented, mean changes would be +0.73°C SST and a reduction to pH 8.0 for surface seawater (IPCC, 2014; Pörtner et al., 2019; IPCC, 2021). Nevertheless, these changes will not occur equally across the globe. As predicted by climate models, higher latitudes will warm faster and experience quicker declines in pH than lower latitudes (Fabry et al., 2009). For example, the North Sea has warmed 1.67°C since 1962, a rate faster than other oceans (Wiltshire et al., 2010).

Human activities that have a direct impact on marine ecosystem are the exploration of the ocean floor in search of natural resources such as petroleum and gas deposits. The increased exploitation of marine resources especially in developed countries (*i.e*. UK, Germany and China) in the last decades has led to more sound-producing activities such as marine traffic, piling, seismic surveys and the building of offshore structures (Duarte et al., 2021). Together changing oceanic conditions and noise pollution are threatening the health of aquatic life. Furthermore, in species with complex life cycles, like the European lobsters, the sensitivity to anthropogenic stressors is particularly high during the larval and juvenile

phase. Thus, a major task for marine scientists in the 21st century is to identify and quantify how multiple environmental stressors may impact key organisms like crustaceans.

1.3.1 Ocean warming and acidification effects on crustaceans

Crustaceans are ubiquitous in the oceans and are exposed to highly diverse and variable environmental conditions. Their tolerance to a wide range of temperature is clear by their global distribution and vast experimental evidence (Hopkin et al., 2006; Schmalenbach and Franke, 2010; Storch et al., 2011; Jost et al., 2012; D'Urban Jackson et al., 2014). However, thermal tolerance can be influenced by abiotic factors like oxygen, pH and salinity. In the face of a climatic crisis provoked by an increase of CO_2 emissions, seawater pH has become one of the main environmental factors to take in consideration in research on climate change. The ocean absorbs approximately one quarter of the anthropogenically released CO_2 (Le Quéré et al., 2016), this results in an increase of free hydrogen ions, thereby lowering the seawater's pH. The net results are changes in seawater CO_2 partial pressure (pCO_2), and [CO_3^{2-}], which translates to a decrease in biologically available carbonate. The reduction in carbonate ions affects marine calcifying organisms that build hard body structures made of calcium carbonate ($CaCO_3$). Therefore, there has been a tendency in research to focus mostly on the biological effects of ocean acidification (OA) on corals, echinoderms and molluscs; even though crustacean's exoskeleton are made of chitin [$(C_8H_{13}O_5N)_n$] which could be affected by changes in seawater carbonate chemistry. There are still knowledge gaps on the potential effects OA will have on crustaceans' fitness, especially studies focusing on the interactions between multiple environmental stressors. Furthermore, more studies are needed on the sensitivity of economically importance species to climate change, that are additionally facing fishing pressures, like clawed lobsters.

Available studies on ocean acidification show crustaceans are one of the most tolerant groups of marine invertebrates (Melzner et al., 2009; Kroeker et al., 2011; Whiteley et al., 2018). This greater resistance to OA is suggested to be due to their efficient buffering mechanism. Buffering is done by maintaining acid-base equilibria through iono-regulation (*i.e.*

in regulation of HCO_{3-}) (Whiteley et al., 1999; Whiteley, 2011). Crustaceans are water breathers and are directly exposed to ocean acidification through their gills which are specialized for respiratory gas and ion exchange (Taylor and Taylor, 1992). Changes in seawater carbonate chemistry lead to an unbalance and interrupt proper CO_2 excretion across the gills causing the accumulation of CO_2 in the haemolymph (Whiteley, 2011). Nevertheless, crustaceans can adjust haemolymph pH through electroneutral ion exchange across the gill epithelial to some extent. (Whiteley et al., 1999). However acute or long-term exposure to OA, interrupts this process, reducing oxygen delivery to tissues (Whiteley and Taylor, 1992; Whiteley et al., 1999). Thus, compromising metabolism, growth and consequently survival.

Responses to environmental stress may vary across life stages and species with the tendency for early life stages to be more sensitive and less tolerant (Kikkawa et al., 2003; Ishimatsu et al., 2004; Kurihara, 2008). Past studies investigating underlying physiological responses to elevated pCO_2 in crustacean larvae show there are energetic trade-offs between physiological processes. Elevated pCO_2 can have an effect on larval metabolism, elemental composition and mineralisation (Arnold et al., 2009; Carter et al., 2013; Schiffer et al., 2013). Furthermore elevated pCO_2 co-occurring with higher temperatures increases an organism's thermal sensitivity by narrowing its aerobic thermal window (Metzger et al., 2007; Pörtner, 2008; Walther et al., 2009). When an organism is exposed to severe heat stress the cost of maintaining physiological demands (*i.e.* metabolism, growth) increases. If the energy available is not enough to cover all those demands, this pushes the organism to a *pejus* limit (Pörtner, 2010). The term *pejus* (Latin: “turning worse”) refers to the point where an organism’s performance begins to decline (Frederich and Pörtner, 2000; Frederich et al., 2009). The *pejus* range is followed by the critical point or *pessimus* (Latin: “worst”) range, when there is a switch towards anaerobic metabolism even if sufficient oxygen is present in the environment and performance declines substantially making recovery unlikely (Jost et al., 2012). Some species, like *Cancer irroratus* and *Homarus americanus* gradually decrease aerobic activity or performance between the *pejus* and *pessimus* temperature. While other species like *Carcinus maenas* decrease aerobic performance rapidly from the optimum to *pessimus* range, without going through a detectable *pejus* threshold (Figure 2; Jost et al.,

2012). The avoidance of going through the *pejus* range may be an adaptive strategy to survive in a highly fluctuating environment as the intertidal zone.

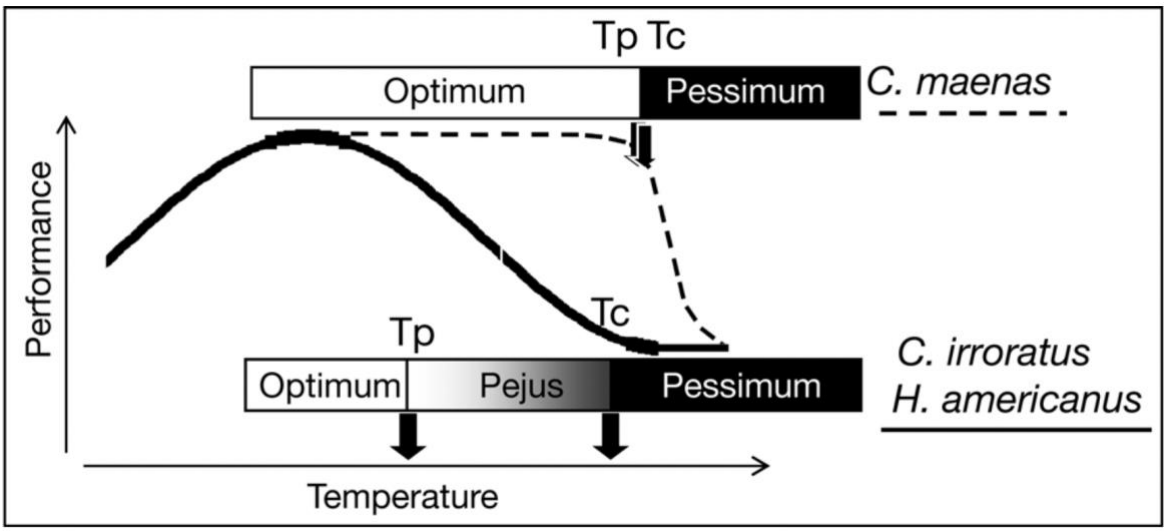

Figure 2. Temperature tolerance in crustaceans (Jost et al., 2012)

Interpreting the joint effect of OA and warming on crustaceans is still challenging, because as mentioned before there appears to be no clear pattern, and responses may be species and stage specific. For example elevated CO_2 concentrations narrowed the thermal tolerance window of *Cancer pagurus* (Metzger et al., 2007), but oxygen consumption rates measured in *H. gammarus* and *H. americanus* larvae show no significant effects of interactions between elevated temperature and pCO_2 (Small et al., 2016; Waller et al., 2017). Similarly, some crustaceans have a higher threshold for acidic waters, like copepods (a negative impact was seen only below 7.0 pH) (Weydmann et al., 2012); while others are more sensitive, like the shrimp *Palaeomon pacificus* (a negative impact was seen at 7.89 and 7.64 pH) (Kurihara, 2008). Moreover, crustaceans' sensitivity towards OA varies withing life stages. Larval and juvenile stages have been reported to be more sensitive to OA than adult individuals (Dupont et al., 2010; Ross et al., 2011). For example the following crustaceans species showed decrease survival in high CO_2 environments: American juvenile lobsters (Menu-Courey et al., 2019), juvenile red king crab (*Paralithodes camtschaticus)* (Long et al., 2013), Dungeness crab larvae (*Cancer magister)* (Miller et al., 2016) and the porcelain crab larvae, *Pethrolisthes cinctipes* (Ceballos-Osuna et al., 2013). Other sub-lethal responses to

climate change include negative impact on cellular respiration, enzyme regulation and metabolism (Metzger et al., 2007; Whiteley, 2011).

Therefore, it's worth investigating the mechanism of tolerance at different levels from the molecular level to the whole animal. There are certain proxies that are regularly used to measure environmental stress. Under suboptimal environmental conditions, reduced larval growth and development are generally the conditions affected first (Walther et al., 2009; Anger et al., 2020). To evaluate the impact ot environmental stress on larval growth the most common indices measured are body size, biomass and elemental compositions (*i.e.* carbon and nitrogen content: (Ritar et al., 2003; Wu et al., 2013; Torres et al., 2021). To explore how an organism is coping under multiple environmental stressors, routine metabolic rates are traditionally investigated as an approach to confirm how elevated CO_2 concentration enhance the sensitivity of organisms to thermal extremes (Storch et al., 2011; Waller et al., 2017; Laubenstein et al., 2019).

Additionally, a way to identify at the cellular level whether an organisms reached the *pejus* or *pessimus* level which translates as a switch from aerobic to anaerobic. Is to measure the by-products of this transition which are an excess of reactive oxygen species (ROS) that lead to oxidative stress. To counteract ROS-induced damage, cells are equipped with antioxidant enzymes like: catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and Glutathione peroxidase (GPx) (Holmblad and Söderhäll, 1999). Therefore, fluctuations of these enzymes' activity can serve as a proxy to identify when an organism's defense mechanism has been compromised or suppressed by environmental stressors (Beliaeff and Burgeot, 2002; Rato et al., 2017; Tremblay et al., 2020).

There is a limitation in predicting how the *Homarus* lobsters will face on going climate changes. Since, to date there are only two studies investigating the joint effect of ocean warming and acidification on lobster larvae. The results of these studies demonstrate elevated temperature has a stronger effect on life history (survival and development) and physiological responses (oxygen consumption rates) of lobster larvae than elevated pCO_2 (Small et al., 2015; Waller et al., 2017). The sensibility of larvae to climate change pinpoints

the need to study early life stages tolerances to environmental stressors in order to avoid the risk of under-estimating the potential ecological consequences on population dynamics (Russell et al., 2012). In the case of Helgoland's lobsters, OA and warming could have particularly strong effects on the recruitment of the remaining natural and program-released specimens.

1.3.2 Noise pollution and crustaceans

The seas are full of sounds: the type that provides crucial information to marine organisms and the added anthropogenic sound. Human produced sounds are considered "noise" from a biological point of view because they are unnatural or unwanted signals in the environment. Anthropogenic noise is increasing at a steadfast rate, due to industrialization (Hildebrand, 2009) and the growing human population which the oceans sustain. Consequently, anthropogenic noise is considered a new source of pollution which is pervasive across all oceans (Peng et al., 2015; Williams et al., 2015). Even though some human activities (i.e fishing, sailing) have existed for centuries; novel anthropogenic noise sources have developed and intensified in approximately the last 50 years (Andrew et al., 2002; Malakoff, 2010). Among these activities, the switch to renewable energy involving offshore windfarms (OWF) and tidal energy has further contributed to noisier waters (Pine et al., 2012). However, the byproduct noise produced by the operation of renewable energy is less intense compared to the deliberate noise in seismic surveys. Which are used to detect the presence of petroleum and gas deposits below the seafloor. Other sources of anthropogenic noise that have been discussed in noise pollution studies include dredging, construction of harbors, bridges, oil and gas platforms, pile driving, shipping, recreational boating, explosives, the use of sonar by commercial and military vessels (Popper et al., 2007; Hildebrand, 2009; Reine et al., 2014; Hawkins et al., 2015; Duarte et al., 2021).The noise human activities produce can be divided in different categories depending on their noise levels and how long they last. These are high-intensity impulsive noise (e.g pile-driving, underwater blasting) and low-frequency stationary noise (e.g tidal and wind turbines) (Peng et al., 2015; Tidau and Briffa, 2016; Duarte et al., 2021).

Sound propagates relatively fast and far underwater, efficiently transmitting signals used by marine organisms to interpret and explore their environment. Sound can be described as a type of mechanical vibration that propagates through fluids in a form of a wave. Underwater sound consists of both pressure waves that can travel through the water and the seafloor (Aimon et al., 2021). The difference in hearing range and how sound is perceived between marine mammals, fishes and marine invertebrates comes from their auditory systems. Aquatic animals, like fish that have gas filled organs detect sound pressure waves when their auditory sensors are squeezed. Crustaceans, however, lack gas filled organs (e.g swim bladders) and therefore use mechanoreceptors like setae (surface sensory hairs) that cover their carapace, antennae and internal statocysts structure to detect vibrations (Popper et al., 2001; Breithaupt, 2002). The statocyst is a mechanosensory organ that allows crustaceans to detect movement, gravity and position (Day et al., 2020). Crustaceans are equipped to sense vibrations and thus are more perceptive to particle motion than sound pressure variations. Hearing curves measured in several species: common prawn (*Palaemon serratus)*, mud crabs (*Paenopus spp),* Norway lobsters (*Nephrops norvegicus)* and American lobster (*Homarus americanus)* show the highest sensitivity for lowest frequency (100 Hz, 75 Hz, 20-200 Hz and 80-120 Hz respectively)(Goodall et al., 1990; Lovell et al., 2005; Hughes et al., 2014; Jézéquel et al., 2021). Furthermore, many aquatic crustaceans are known to produce a variety of sound through different mechanisms like “carapace vibration” in clawed lobsters (Henninger and Watson, 2005; Jézéquel et al., 2018), “cavitation bubble collapse” in snapping shrimp (Knowlton and Moulton, 1963) and “stick and slide friction” in spiny lobster (Patek, 2001). The biological significance for these sounds is still unclear for some species. Some suggested hypotheses are for antipredator defense in palinurids (Bouwma and Herrnkind, 2009; Buscaino et al., 2011) and to deter predators and signal conspecifics of their presence in territorial European lobsters (Jézéquel et al., 2018). From previous studies on sound characterization of crustaceans it can be concluded sounds produced, whether addressed to predators or conspecific, are an advantage to crustaceans since they can acquire information about a potential threat and their surroundings

Unfortunately, anthropogenic noise has become an obstacle by masking the sounds marine life use for communication (Hawkins et al., 2015; de Jong et al., 2018). These leads to the loss of vital information and is one of the various ways noise pollution is negatively affecting marine life. Based on the existing studies on the impacts on aquatic noise on crustaceans (Peng et al., 2015; Edmonds et al., 2016; Tidau and Briffa, 2016) , negative effects of anthropogenic noise on crustaceans can be divided into (1) acoustic masking, (2) physiological damage to hearing system, (3) other physiological impacts and (4) behavior alteration. Compared to vertebrates the effect of direct damage on the hearing system of invertebrates remains poorly understood. However, a study done by Day et al., (2019) was the first to demonstrated exposure to seismic air gun signals caused morphological damage to the statocysts of rock lobsters (*Jasus edwardsii*) and consequently impaired their righting reflex. Furthermore, noise can lead to a series of physiological stress responses. In a study by Wale et al., (2013), the green crab (*Carcinus maenas*) consumed more oxygen when exposed to ship-noise than those exposed to ambient-noise. Similarly, noise exposure in the brown shrimp (*Crangon crangon*) led to an increase in oxygen consumption and slower growth rate associated with noise-induced stress (Lagardère, 1982; Regnault and Lagardere, 1983). Other stress-related biochemical adjustments have been identified in crayfish (*Procambarus clarkia*) and European spiny lobsters (*Palinurus elephas*) when exposed to vessel noise. These changes include a decrease in total haemocyte count (THC) and an increase in heat shock protein 27 (Hsp27) expression and glucose levels (Celi et al., 2012, 2015; Filiciotto et al., 2014). In addition, anthropogenic noise can alter key behaviors in crustaceans such as: foraging, shell searching behavior, shelter preference, grouping behaviors and antipredator responses (Wale et al., 2013a; Roberts and Laidre, 2019; Tidau and Briffa, 2019, 2019). The latter can be explained by the "distracted prey hypothesis, which implies anthropogenic noise may reallocate the organism's attention, distracting them, and therefore preventing them to respond to predatory threats (Chan et al., 2010).

Given the harmful impacts or anthropogenic noise on crustaceans, it is important to determine all sources and more importantly how long-term exposure to noise can affect their choices and ecosystem. Marine noise pollution frequently focuses on the effect of short-lived intense noise (i.e. seismic surveys, underwater constructions) on marine taxa. However,

considering there are long lasting projects like offshore OWF which produce chronic low-intensity operational noise possible for decades. It is essential to have more research on how marine life like decapods living among their foundation will be affected by constant noise exposure. Thus, anthropogenic noise risk assessments ought to include frequently overlooked benthic communities. Moreover, current knowledge of how underwater noise may synergize with other abiotic (e.g. ocean acidification) and biotic (e.g. predation) stressors is limited.

Aims of the thesis

The overall aim of the thesis was to investigate the influence of anthropogenic stressors on the recruitment success of Helgoland's lobster larvae in order to contribute to the protection of lobster population in the southern North Sea. Specifically, I focus on the effects of noise pollution, warming and acidification on lobster's early life stages.

The specific aims of this thesis are:

1. Identify a way of successfully monitoring and sampling lobster larvae on Helgoland.
2. Determine how ocean warming and acidification affect lobster larvae.
3. Investigate the effect of anthropogenic noise on juvenile lobster behavior.

To achieve these specific aims, different techniques and approaches were used. These include field and extensive laboratory work as well as two factorial experiments with multiple biotic (*i.e*. predator presence) and abiotic stressors (i.e. temperature, acidification, noise).

In **chapter two,** I present the catch efficiency of a modified light trap for lobster larvae. Lobster traps were evaluated in the laboratory using different water volumes, larvae densities and light sources. Then the traps were tested in the field during lobster hatching season (May to August). These experiments aim to draw attention to the low number of lobster larvae in the field and the challenges that persist to date in catching larvae.

In **chapter three,** I assess the effects of both ocean warming and acidification on the growth, survival and fitness of lobster larvae. Larvae were reared from stage I to stage III under a gradient of ten seawater temperatures combined with moderate and elevated seawater pCO_2 treatments. Using a large temperature gradient, I expect to have more precise insight on the aerobic thermal window trade-offs of lobster larvae. My hypothesis is that exposure to ocean acidification will narrow the larvae thermal tolerance. These would lead to a negative effect on growth, development and metabolism.

In **chapter four,** I investigated the effects a low-frequency constant noise has on juvenile European lobster antipredator behavior and substrate choice. This experiment aims to understand how noise may synergize with other stressors. Juvenile lobsters were exposed to both noise and/or predator presence in a diurnal and nocturnal experiment. Lobsters were filmed and then I analyzed their behavior to understand the impact noise had on their decision-making process (choosing rocks or oyster as shelter) as well as their locomotor activity. I expect noise would modify the way lobsters react to predators in comparison with lobsters in control treatments.

Lastly in chapter **five,** I integrate the findings of the different experiments in a broader perspective and give my opinion on future research directions. I discuss how European lobsters are coping with changing oceanic conditions and re-evaluate Helgoland's lobster conservation efforts.

Publications and manuscripts

The thesis is based on the following papers:

Paper I (Chapter 2)	**Leiva L**, Giménez L, Boersma M. Evaluation of light traps for sampling European lobster larvae in the German Bight, North Sea. *To be submitted to Marine Biology.*

Paper II (Chapter 3)	**Leiva L**, Tremblay N, Torres G, Boersma M, Krone R, Giménez L. (2022) European Lobster Larvae Development and Fitness Under a Temperature Gradient and Ocean Acidification. Frontiers in Physiology 13, 809929. doi.org/10.3389/fphys.2022.809929
Paper III (Chapter 4)	**Leiva L**, Scholz S, Giménez L, Boersma M, Torres G, Krone R, Tremblay N. (2021) Noisy waters can influence young-of-year lobsters' substrate choice and their antipredatory responses. Environmental Pollution 291, 118108. doi: 10.1016/j.envpol.2021.118108.

My contributions to the papers:

Paper I: The scientific concept of this manuscript was developed by me in discussion with M. Boersma and L. Giménez. Sampling of lobster larvae, experimental and analytical work, the evaluation of the data and the writing of the manuscript was done by me.

Paper II: The scientific concept was developed by N. Tremblay, G. Torres, M. Boersma, L. Giménez and me. I performed the experimental and analytical work with the support of N. Tremblay. Data analysis was done by me, N. Tremblay and L. Giménez. I wrote the manuscript, which was commented and improved by the co-authors.

Paper III: The scientific concept of this manuscript was developed by me and N. Tremblay, in discussion with M. Boersma. Experimental and analytical work as well as the evaluation of the data was performed by me, S. Scholz and N. Tremblay. Statistical analyses were done by me with the help of L. Giménez. I wrote the manuscript, which was commented and improved by the co-authors.

CHAPTER 2. EVALUATION OF LIGHT TRAPS FOR SAMPLING LOBSTER LARVAE IN THE GERMAN BIGHT, NORTH SEA

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Manuscript

Abstract

Biological monitoring of planktonic animals is greatly dependent on the deployment of traps. A variety of specialized traps have been designed for surface plankton and vertebrates. However, some groups like benthic marine invertebrates remain under sampled. Catching them has proven to be more challenging because of their size, swimming ability, location and abundance. In the present study a successful light trap for sampling American lobster larvae in New Brunswick, Canada is evaluated on the island of Helgoland (German Bight, North Sea). Our results showed the traps were successful in catching larvae in laboratory experiments but were unable to catch European lobster larvae in the field. Traps deployed in the field were successful in capturing other benthic and pelagic zooplankton predominantly consisting of crustaceans from the orders: Cumacea, Amphipoda, Mysida and Isopoda. The low density of lobster larvae, the island's topography and their unique photactic response possibly limited the success rate of the light traps. Future research is needed to

construct a specialized trap to sample Helgoland's lobster larvae and provide information on the current larval fitness and population numbers.

2.1 Introduction

For many marine benthic species, the larval phase is the principal dispersal stage, as the planktonic larvae remain in the water column until equipped to settle and metamorphose. This means that for the assessment of reproductive effort it is important to determine larval densities but selecting the correct method to sample them can be challenging. Sampling of larvae during this stage for biological monitoring is predominantly done by net tows and/or passive collectors. Larval nets vary in mesh size and opening as well as in the direction of the pull which can be horizontal or vertical. Passive collectors principally lure larvae with light, and the traps used vary mostly in the intensity of the light and the shape of the opening. These commonly used methods have their advantages and disadvantages related to deployment, efficiency to catch certain taxa and the preservation of the caught organisms (McLeod and Costello, 2017). Larval tows are weather-dependent and difficult to deploy in shallow areas bordering rocky coastlines. Therefore, they involve more logistical planning and thus are more expensive if multiple locations are needed to be sampled, contrary to passive collectors such as light traps, which are more economical to build and thus can be deployed for multiple days at different sites using smaller vessels (Øresland, 2007). Moreover, the selectivity of these methods is very different. Larval tows are efficient at sampling slow and abundant surface planktonic organisms (Øresland, 2007; Pineda et al., 2010; Sigurdsson et al., 2014), whereas passive collectors can be more efficient at sampling strong-swimming and scarce organisms, such as crustacean larvae (Sigurdsson et al., 2014).

The distribution of larvae in the water column is dependent on various environmental factors like water depth, currents, temperature, food abundance and the light-dark regime. These factors play an important role in providing cues on orientation and depth regulation of planktonic larvae (Forward, 1974, 1989). Accordingly, benthic and pelagic zooplankton (including larvae of invertebrates and fish) are often positively phototactic. The attraction to

light has facilitated the capture of zooplankton by using light to lure animals into traps (Floyd et al., 1984; Chan et al., 2016; McLeod and Costello, 2017). Catching larvae in the field and monitoring serves as an instrument to draw conclusions on the current population numbers of a particular species and its vertical distribution. The number of competent larvae caught in larval tows or traps is key to correlate planktonic larvae supply, recruitment success and benthic community composition (Gaines et al., 1985). As mortality is high during this stage, assessing stocks in the wild is important, also in the context of management of species that are vulnerable. Marine decapods like lobsters, which are heavily fished in the wild are often in need of monitoring. Since they have complex life stages, it is necessary to understand if there might be bottlenecks at the larval stage, potentially hindering recruitment.

Larval tows have been used to quantify the abundance of different larval stages of the American lobsters since the 1930s (Fogarty, 1983). This method captures relatively high numbers of stage I larvae, but lower numbers of stage III and III, and even fewer stage IV larvae (Wilder, 1953; Scarratt, 1973). The possible limitation larval tows may have in catching larvae in more advance stages is that as larvae metamorphize they become stronger swimmers and may avoid nets (Fleminger and Clutter, 1965). Furthermore, wave actions and rain can cause larvae that are usually found in surface layers to move to deeper waters (Wilder, 1953; Øresland, 2007). In the last decades several studies have shown light traps can complement larval tows (Doherty, 1987; Meekan et al., 2000; Mwaluma et al., 2009). Light traps have become an additional and successful tool for vertical sampling of larvae from the *Homarus* and *Nephrops* genera (Øresland, 2007; Sigurdsson et al., 2014). The main argument for their usage and popularity as a sampling tool for pelagic larvae, is that light traps require less working hours, and catch rates are comparable to larval tows (Øresland, 2007). Other advantages are that animals caught are in good conditions and therefore can be used in experiments and morphological work.

Knowledge of the vertical distribution of the European lobster larvae population on the island of Helgoland is absent. To date no sampling method has been successful in catching lobster larvae around the island of Helgoland. In Germany the European lobsters are restricted to the island of Helgoland (German Bight, North Sea) and the local lobster

population remains at critically low levels due to overfishing and habitat destruction (Anger and Harms, 1994; Franke and Gutow, 2004). Annual landings are currently only a few hundred lobsters per year in comparison to a yield of 38 tons per year in the 1920s-30s (Klimpel, 1965; Schmalenbach and Buchholz, 2010). The second indication of very low adult densities are the low numbers of lobster larvae found in the field (Greve et al., 2004). Lobster larvae are rarely found in the Alfred-Wegener-Institut Helmholtz-Zentrum für Polar-und Meeresforschung (AWI) long time-series plankton sampling (Helgoland Roads) CalCOFI net hauls. In fact, lobster larvae have only been found two times in the sampling period between 1975 (0.03 ind/m^3) and 2018 (0.02 ind/m^3). Thus, the question remains whether the adult spawning densities are so low that it is numerically unlikely to catch larvae, or spawning takes place farther away from the island of Helgoland, or whether the strong tidal currents and wind impact carry the larvae further into the open sea. Moreover, it could be a certain behavior (e.g. migrating immediately to the seabed) that prevents larvae from being caught with plankton hauls.

The sampling device we chose to evaluate was the "tube light trap" which has been successful in catching *H.* americanus in New-Brunswick (Canada), and *H. gammarus* and *Nephrops norvegicus* larvae in Sweden (Øresland, 2007; Sigurdsson et al., 2014). Additionally, we chose this design because the trap meets the following criteria: (1) robust; (2) economical; (3) able to sample multiple locations simultaneously; (4) easy emptying without loss of animals; (5) allows sampling while drifting with the currents; (6) permits sampling for up to 24 hours without reduction in light intensity.

The present study assessed the efficiency of a modified light trap in the laboratory, and in the field at different depths to gather more information on lobster larvae distribution. The main objective of our study was to evaluate the potential of using light-traps on the island of Helgoland to support research on the European lobster in the wild and conservation actions. We were particularly interested in capturing larvae to assess the larval fitness, the current potential for settlement in the area, and in the future use of light traps as a tool for studies on connectivity and recruitment. Additionally, through our experiments we wanted to test and improve a low-cost, robust and eco-friendly light trap for laboratory and field experiments for crustacean larvae.

2.2 Materials and methods

Origin of animals

The study was carried out at the at Alfred-Wegener-Institut Helmholtz-Zentrum für Polar-und Meeresforschung (Helgoland, Germany). European lobster larvae (*Homarus gammarus)* were borrowed from the lobster-rearing facilities for lobster conservation, Reefauna. Larvae hatched from ovigerous female lobsters captured by local fishermen in the rocky subtidal zone around the island of Helgoland (German Bight, North Sea, 54°11:3'N, 7°54.0'E).

Design and construction of trap

We use a slightly modified version of Sigurdsson (et al., 2014) light trap. Our version consists of a funnel shaped glass with a wider entrance, a narrower opening (1 cm diameter orifice) and the closed end is shut with a removable plastic lid. The body of the trap is made of a red PVC pipe, 10 cm in diameter and 40 cm long. The plastic lid was fitted inside with a ring where the chemical lights or LED lights could easily be secured and removed after use (Figure 3). For deployment two stainless steel rings were attached to the traps one at the closed end and the other side. In order to avoid loss of caught organisms through the entrance when traps were pulled vertically; a rope was passed through the top ring to lower the traps into the water columns, and for retrieval the traps were pulled by a rope passing through the side rings. Yellow chemical lights (e.g glow sticks, [6 inch, SnapLight Cyalume Technologies, USA]) or white LED lights (PotLight, Fishtek Marine, UK) were used to lure the lobster larvae into the traps. Light sources used in our light traps are in the range of wavelengths (380 – 750 nm) shown to provoke a phototactic response in other crustacean larvae (Forward, 1974; Schmalenbach and Buchholz, 2010). The yellow chemical lights have a wavelength of around 580 nm, and LED white light have wavelength of around 500 nm.

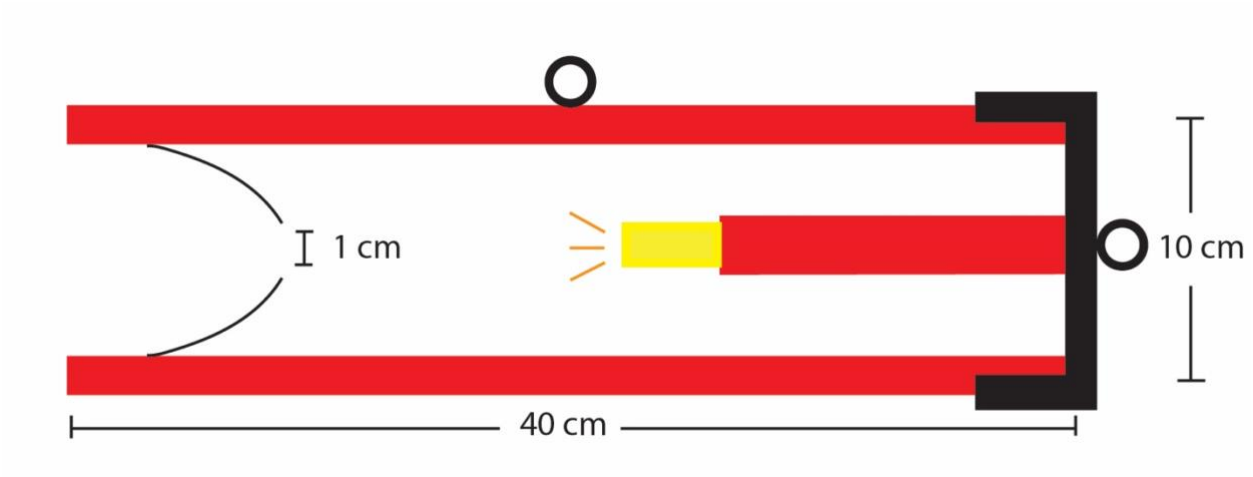

Figure 3. Diagram of the light trap used in the study. The main body of the trap was made of a red PVC tube. The opening was made of glass and the trap was sealed with a plastic cap. Attached to the plastic cap was a fitting for the lights (shown in yellow).

Laboratory experiment in small volume

To test if recently hatched larvae were lured into the light traps, two different light sources as lures were examined: (1) yellow chemical lights and (2) white LED lights. Traps were placed in plastic tanks (49 cm diameter, 72 cm high), containing ca. 100 L of seawater at a controlled temperature of 18°C. The traps were placed approximately 50 cm from the bottom of the tanks. Seawater was gently bubbled with air throughout the experimental runs. The experiment was done in a controlled temperature and light room (12h light/dark). Ten recently hatched lobster larvae (1 and 2 days since hatching) were randomly selected and released in each of the four tanks containing one light trap. Two of the tanks had traps with chemical lights while the other two had traps with LED lights. The experiment ran from 17:00 h to 9:00 h, at the end of experimental run, catchability rate was determined by how many larvae were inside of the trap. The experiment was repeated 28 times (n=280).

Laboratory experiment in large volume

The second laboratory experiment was done in July 2020, in a lower larvae density setting (10 ind/m^3 but in much larger experimental containers, comparing two different light sources as lures. We again used yellow chemical lights and white LED lights. The tanks used

were located outside the facilities of the AWI Helgoland Marine Station. The tanks were made of black plastic and had a cover to prevent light from entering. Two traps with different light sources were placed per plastic tank (256 cm diameter, 110 cm high) containing ca. 5000 liters of fresh seawater directly pumped from the North Sea at a temperature of 18.3 ± 0.14°C. Traps were placed approximately 50 cm from the bottom of the tanks. Fifty recently hatched larvae were released in each tank and left overnight for 16 hours (17:00 - 9:00 h). The following day larvae were counted and retrieved from the traps and tanks. The tanks were emptied and refilled with fresh seawater in between runs, a total of 8 runs were done (n=800). After each experimental run, larvae removed from tanks and traps were returned to Reefauna.

Field sampling

A total of six light traps were deployed in two different sites (Figure 4) in the northern and southern part of the island of Helgoland: Nordmöle (54°11:434'N, 7°52:493'E; depth: 3.2 m) and Nebelhorn (54°10:343'N, 7°53.949'E; depth 9.2 m) respectively. The areas were chosen based on high adult lobster densities as recommended by local sailors and adequate lobster habitat areas which consist of a rocky subtidal zone. In each site, three traps were deployed on an anchored line, buoyed at the surface. At the Nordmöle three traps anchored to a 10 m rope, were tied at 2 m, 4 m and 6 m from the seafloor. At Nebelhorn one trap was attached per rope at a depth of 1 m. The light traps were spread so they were approximately 1.5 m apart from each other. The differences in trap depth deployment are due to the site's seafloor depths, such that the distances to the sediment were similar. Traps were deployed once a week from May to August 2020. They were deployed at high tide in the daytime and left overnight for retrieval the next day during high tide. When the traps were retrieved, they were carefully pulled out of the water with the opening pointing up, to avoid loss of larvae. The traps were then immediately placed in individual buckets with seawater and transported to the AWI Helgoland Marine Station facilities for identification of the catch. Presence or absence of lobster larvae was recorded and additionally all other organisms captured by the traps were counted and grouped by order and identified to family or species level when possible.

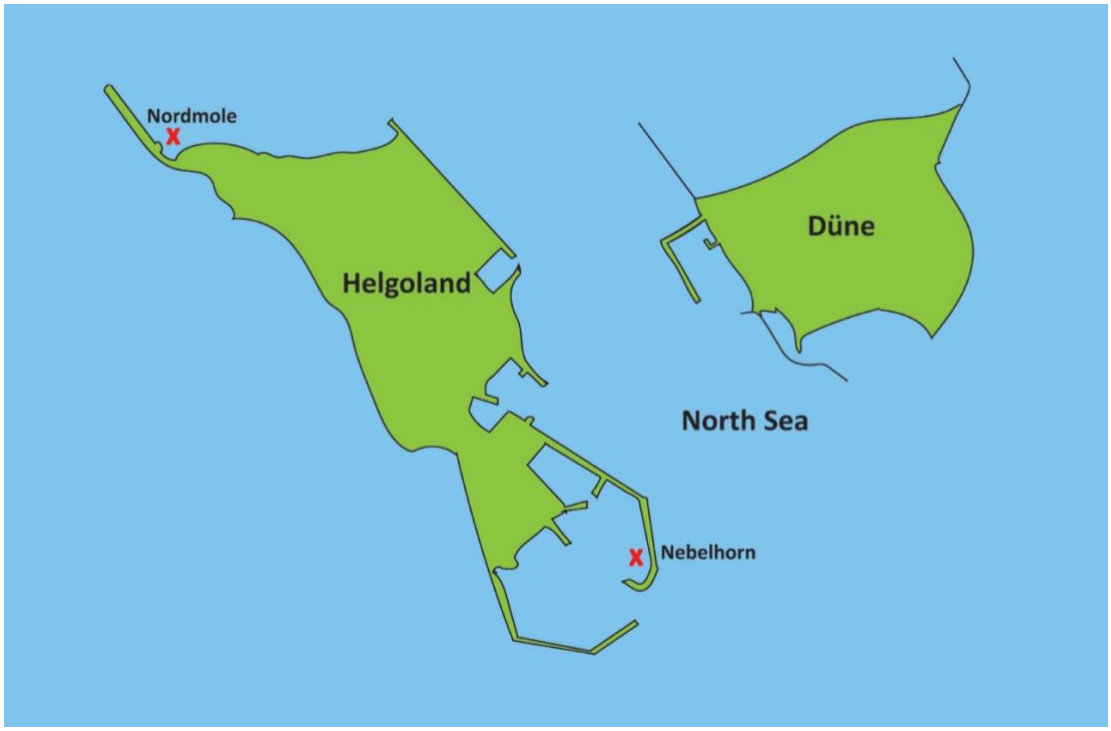

Figure 4. Deployment trap locations on the island of Helgoland. Traps were deployed in the in the North at the Nordmole and in South at Nebelhorn, sites are marked with an (X).

Data analysis

Statistical analyses were performed in RStudio (2022). Data was tested for normality and variance homogeneity using the Shapiro- Wilk and Barlett's test respectively. Difference between light treatments was then tested using analysis of variance (two-way ANOVA). If criteria of a normal distribution or variance homogeneity were not met, the non-parametric Wilcoxon signed rank test was performed. For all the statistical tests, significant difference was set at $p < 0.05$. For the comparison of larvae caught by LED or chemical light traps the chi-squared test was used in the analysis of contingency tables based on the counts of larvae entering traps with LED or chemical lights.

2.3 Results

Laboratory experiment in small volume

There was a marginal significant difference in capture rates between the two light types used in the traps (p-value=0.056). The percentage of larvae captured after 24 hours varied between 42-60 %. Traps using LED lights had a higher catch (mean ± SD, 60.0 ± 16.9%) in comparison to chemical lights (42.5 ± 15.6%) (Figure 5).

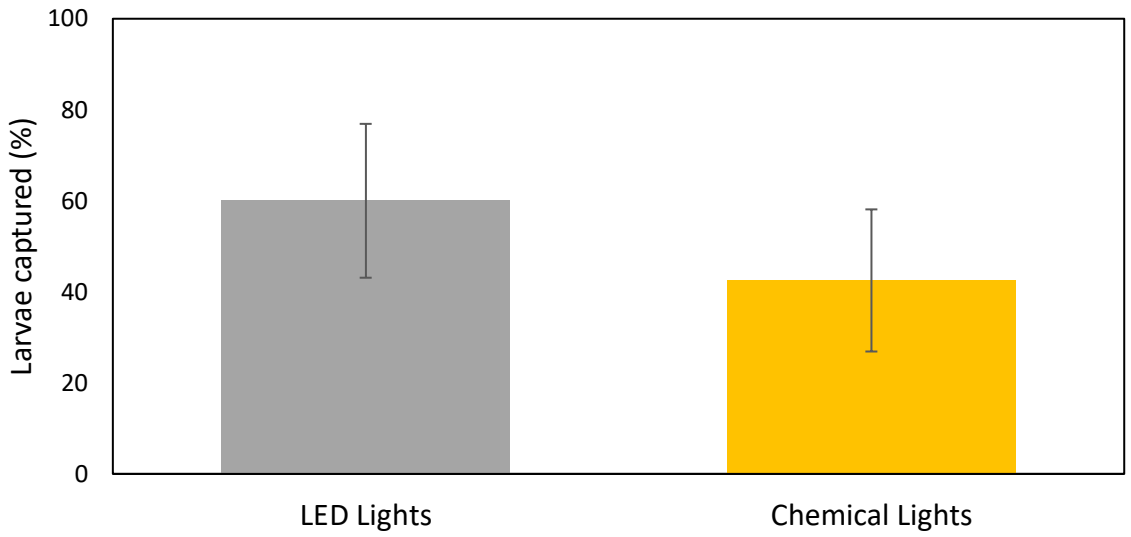

Figure 5. Percentage of *Homarus gammarus* larvae captured by the different light sources after 24 hours.

Laboratory experiment in large volume

Light source has a significant effect on the number of larvae entering each trap (chi-square test, p-value= 2.2e-16). Overall, 135 lobsters were captured by traps with LED light and 11 lobsters were caught by traps using chemical lights. The highest percentage of larvae caught during an experimental run was 46% by an LED light trap and 4% by chemical light trap. While the lowest capture percantage for LED light trap and chemical light trap was 24% and 0% respectively (Figure 6).

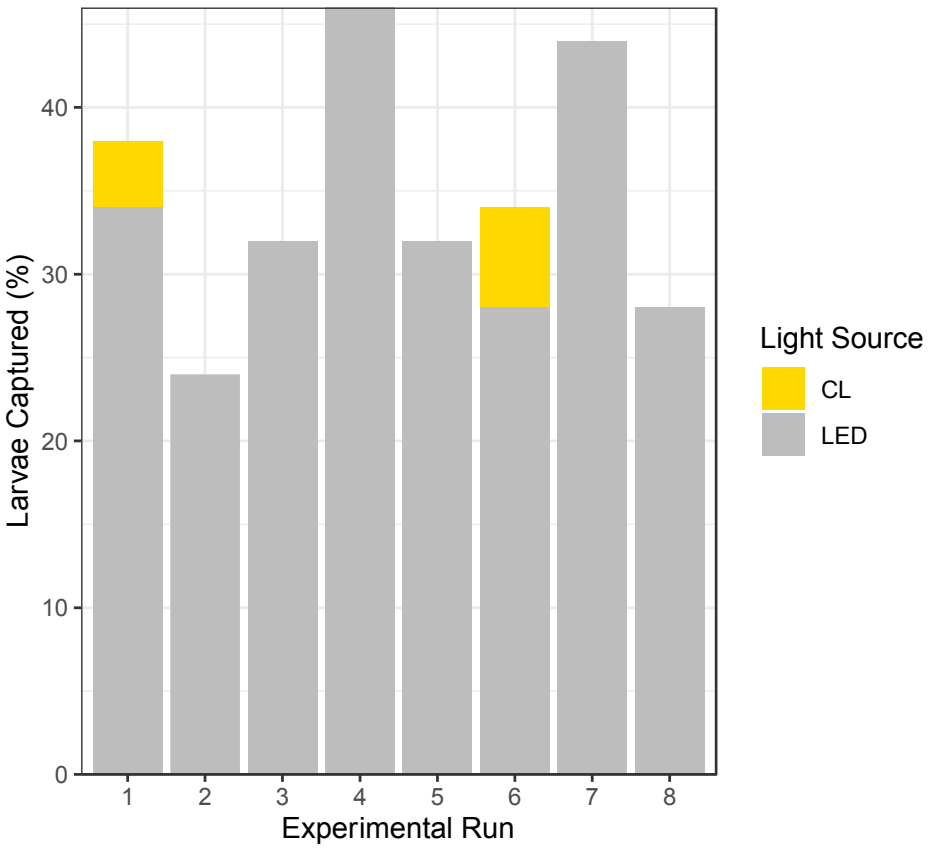

Figure 6. Comparison of light sources efficiency in catching *Homarus gammarus* larvae. CL= chemical lights and LED= LED lights.

Field sampling

No lobster larvae were caught; however, the traps were successful in catching a variety of other small crustaceans. The results of our experiments show that the light traps caught a variety of organisms, and up to 1000 animals in one night (Table 1). Specimens from the following orders were captured: Cumacea, Amphipoda, Isopoda, Decapoda, Trochida, Mysida, Euphausiacea and Cydippida. By far the largest part of the animals caught were Cumacea, followed by Amphipods and Isopods (i.e. family Idoteidae) (Figure 7).

Table 1. Identification and number of specimens collected in the field study (May – August 2020).

Location	Nordmole	Nordmole	Nordmole	Nordmole	Nordmole	Nordmole	Nordmole	Nordmole	Nordmole	Nordmole	Nordmole	Nordmole	
Date	06.05.20	13.05.20	10.06.20	17.06.20	24.06.20	01.07.20	08.07.20	15.07.20	22.07.20	29.07.20	05.08.20	19.08.20	Total
Amphipoda	3		210	8	13	9	35	18	20	25	100	190	631
Cumacea	1	3	2	710	24	25	120	115	400	29	30	900	2359
Decapoda:													
Shrimp	1		2				1				1	1	6
Crab				1			1			4			6
Euphausiacea		3											3
Gastropoda							2	2	11		3	1	19
Fish						1	1					2	3
Mysida					1	5		3		5		1	15
Isopoda									1	2	65	5	73
Cydipidda										2			2
Location	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	Nebelhorn	
Date	06.05.20	13.05.20	10.06.20	17.06.20	24.06.20	01.07.20	08.07.20	15.07.20	22.07.20	29.07.20	05.08.20	19.08.20	Total
Amphipoda		2	15	7	10	9	7	3	1	60	42	29	185
Cumacea			3	2	21	1	8	10	3	60	8	213	329
Decapoda:													
Shrimp										2			2
Crab			1	1			8						10
Euphausiacea													0
Gastropoda					6	1				2			9
Fish					1		1			1			2
Mysida										14			14
Isopoda										130	36		166
Cydipidda													

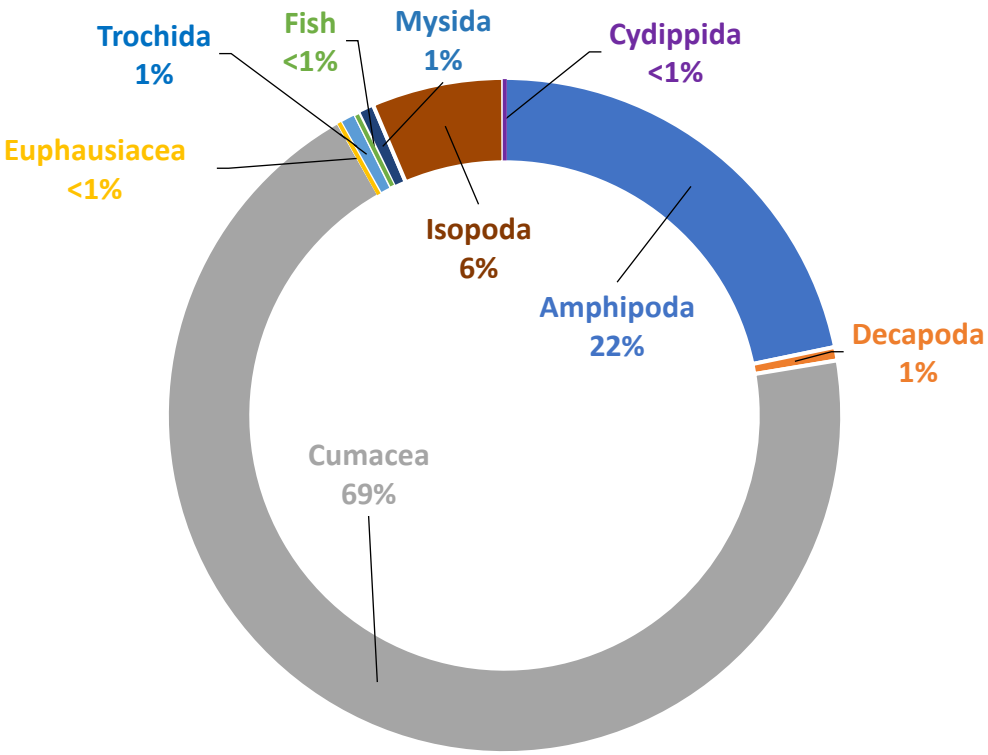

Figure 7. Summary of catch in the traps between the months of May – August 2020.

Several species from the Actinopterygii class (grouped and referred as fish throughout the study) were caught which included two juvenile lumpsucker (*Cyclopterus lumpus*), one sea stickleback (*Spninachia spinachia*), two sand eel (order: Trachiniformes) and a goby larva (order: Gobiiformes). The Decapoda catch was comprised by shrimp and Brachyuran crabs (i.e. *Necora puber*; juvenile and megalopa). Decapod larvae made up 1% of the total organisms caught.

All animals caught were found alive and in good condition. The dominance of Cumacea was seen through the whole summer season (Figure 8); and August was the month with the highest catch coinciding with an increase in both Amphipods and Isopods.

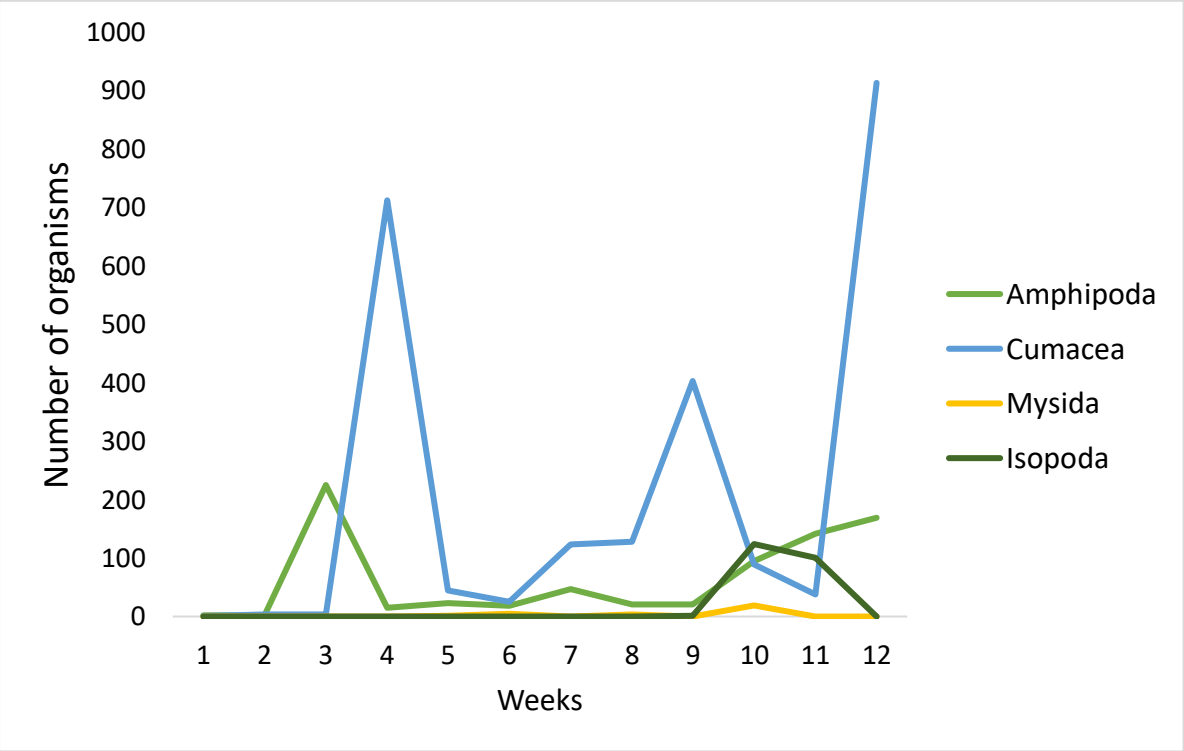

Figure 8. Distribution of most frequently caught organisms in the field. Sampling took place during the months of May (week 1-2), June (week 3-5), July (week 6-8) and August (week 9-12).

2.4 Discussion

It was not possible to catch lobsters in the field using light traps despite intensive efforts throughout the lobster larvae hatching season. A possible explanation for why catching European lobster larvae around the island of Helgoland remains very difficult may be a distinctive negative phototactic response at early larval stages. A study carried out by Schmalenbach and Buchholz (2010), on the vertical positioning and swimming performance of lobster larvae at Helgoland, showed that larvae had a marked positive response to light only at stage I. Moreover, with progressing larval age, the response to light decreases rapidly. Unlike *H. americanus* larvae which have been reported to be negatively phototactic in stage II and III, but again become positively phototactic shortly (~one day) before molting (Hadley, 1908). Another study on *H. americanus* reported early stage IV larvae to be highly photopositive, leading them to illuminated areas in the water column where planktonic food is expected to be more abundant (Botero and Atema, 1982). Observations of European lobster larvae positive attraction to light throughout all larval stages are limited. However a field study by Dunn and Shelton (1983) in Loch Ewe on the West Coast of Scotland noted that around dawn and dusk there was an aggregation of larval stages (I-III) in the upper three meters of the water column. Nevertheless, there is no confirmation that this vertical migration within the upper three meters was in response to light intensity or due to calmer conditions at dawn and dusk. Biological reasons for Helgoland's European lobster larvae strong positive response to light in the first stage may be a way to promote dispersal throughout the rocky bottom around the island. While the abrupt and early change in larval phototactic response to light may prevent larvae from drifting away from the suitable environment (Schmalenbach and Buchholz, 2010). This behavior combined with low lobster population numbers are potentially the reasons why catching lobster larvae around Helgoland remains a challenge.

As there is no current available information on lobster larvae densities around the island. The lobster larvae population on the island of Helgoland has only been calculated based on the density of adult females. There is an estimate of about 15,000 animals around Helgoland on 30 km^2, at a mean water depth of 4 m (Schmalenbach et al., 2011), each producing 20,000 eggs (Coleman et al., 2019). This would mean there should be about 2.5 larvae per m^3 if the larvae were equally distributed and 100% of the eggs survived. However, this is not the case as egg loss in *H. gammarus* has been estimated to be as high as 44% from initial extrusion to hatching (Coleman et al., 2019). To date only three lobster larvae have been caught throughout the Helgoland time-series plankton hauls in the years 1975, 2018 and 2020 at densities of 0.03 ind/m^3 , 0.02 ind/m^3 and 0.04 ind/m^3 respectively. The lack of successful lobster larvae catches in the field make it challenging to have updated larval densities, and at the moment there is a mismatch between estimates and larvae catches.

Helgoland is exposed to strong tidal currents and wind impact, which may lead to variation in current speeds and water level (Schmalenbach and Buchholz, 2010). The chosen deployment sites at the Nordmole and Nebelhorn are protected to a certain extent from strong current that may carry lobster larvae away. Our field experiments results showed traps deployed at both sites caught high numbers of epibenthic organisms, including decapod larvae of other species. However, a higher number of animals were caught at the northern part of the island at the Nordmole. Traps deployed at this site were approximately 1–2 meters from the surface. The depth range at which European lobster larvae have typically been caught and seen (Dunn and Shelton, 1983; Nichols and Lovewell, 1987). Furthermore, the light tube trap design by (Sigurdsson et al., 2014) from which our traps was based, successfully caught *H. americanus* larvae at a depth of 1 m from the surface. Sigurdsson et al., (2014) light traps caught 23 larvae (15 stage I larvae and 8 stage IV larvae) in 281-trap hauls. However, when comparing catch rates and human work hours, the light traps performed similarly to larval tows. Thus, light traps remain a tool that can complement larval tows, especially when aiming to sample and monitor many areas over long periods.

Our laboratory experiment results showed our light traps were capable of luring and capturing larvae in different volumes and thus larval densities. These experiments only

focused on the first larval stage, since as mentioned this is the most positive phototactic stage. In the field where a mixture of larvae between stage I to IV should be in the water column, we expected to catch a mix of larvae at different stages with a majority of early larval stages. The light sources used in our traps influenced the larvae capture rates and overall trap success. White LED lights captured more larvae than yellow chemical lights in the laboratory experiments overnight. As past light trap studies suggest using a light source like LED appear to be the best option as they are robust, long lasting and more ecological (Sigurdsson et al., 2014; McLeod and Costello, 2017). The LED light we used had an intensity of 1.3 lumen and was successful at catching larvae. Sigurdsson et al. (2014) compared capture rates between traps using different LED light intensities of 100 and 4 lumen, and the captures rated between light intensities did not differ significantly. The LED lights used in our experiments used re-chargeable AA batteries, and base con preliminary testes there was no reduction in light intensity after 72 hours. Thus, we suggest using reusable white LED lights (PotLight, Fishtek Marine, UK) instead of single use chemical lights for light trap sampling.

Sampling times and duration are likely to affect catch compositions. Our traps were left overnight and possibly extending this time could have increased our chances of catching lobster larvae. However, leaving the traps for longer periods may also increase predation rates inside the traps. The risk decapod larvae are eaten by predators in our traps is low based on the few predators caught by our traps (e.g fish larvae). In a field study at the Bay of Fundy, Canada light traps were attended every 6-10 days and succeeded in catching *H. americanus* larvae (Sigurdsson et al., 2014). While leaving traps overnight (24 h) also successfully caught 32 stage I and 2 stage II *H. gammarus* larvae in Kåvra, Sweden (Øresland, 2007). Therefore, we suggest experiments should be conducted to compare the catch of the traps over 24 hrs to 10 days; to assess the optimal sampling duration. Our light trap was constructed and modified based on the suggestions provided by (Sigurdsson et al., 2014). We made the entry point (opening) of the traps smaller, by decreasing the size from 24 mm to 10 mm, since lobster postlarvae measure ca. 2-3 mm. Nonetheless, our traps did not catch lobster larvae; but the trap's design proved to be adequate for capturing a variety of benthic and pelagic zooplankton.

To conclude, this study reports the first attempt to catch European lobster larvae around Helgoland, North Sea using and inexpensive constructed light trap. However, low number of adult lobsters around the island combined with and early negative response to light may be the major reasons why catching lobster larvae in the field is challenging. Nevertheless, tube light traps from this study can be used for vertical sampling of a variety of small crustaceans. Especially cumaceans, amphipods and isopods which have a strong response to white LED light and can be easily captured by light traps during the summer season.

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CHAPTER 3. EUROPEAN LOBSTER LARVAL DEVELOPMENT AND FITNESS UNDER A TEMPERATURE GRADIENT AND OCEAN ACIDIFICATION

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European Lobster Larval Development and Fitness Under a Temperature Gradient and Ocean Acidification

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Climate change combined with anthropogenic stressors (e.g. overfishing, habitat destruction) may have particularly strong effects on threatened populations of coastal invertebrates. The collapse of the population of European lobster (*Homarus gammarus*) around Helgoland constitutes a good example and prompted a large-scale restocking program. The question arises if recruitment of remaining natural individuals and program-released specimens could be stunted by ongoing climate change. We examined the joint effect of ocean warming and acidification on survival, development, morphology, energy metabolism and enzymatic antioxidant activity of the larval stages of the European lobster. Larvae from four independent hatches were reared from stage I to III under a gradient of 10 seawater temperatures (13–24°C) combined with moderate (~470 µatm) and elevated (~1160 µatm) seawater pCO_2 treatments. Those treatments correspond to the shared socio-economic pathways (SSP), SSP1-2.6 and SSP5-8.5 (i.e. the low and the very high greenhouse gas emissions respectively) projected for 2100 by the Intergovernmental Panel on Climate Change. Larvae under the elevated pCO_2 treatment had not only lower survival rates, but also significantly smaller rostrum length. However, temperature was the main driver of energy demands with increased oxygen consumption rates and elemental C:N ratio towards warmer temperatures, with a reducing effect on development time. Using this large temperature gradient, we provide a more precise insight on the aerobic thermal window trade-offs of lobster larvae and whether exposure to the worst hypercapnia scenario may narrow it. This may have repercussions on the recruitment of the remaining natural and program-released specimens and thus, in the enhancement success of future lobster stocks.

Keywords: climate change, ocean warming, thermal tolerance, early life stages, decapod

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INTRODUCTION

Since the industrial age, the burning of fossil fuels has led to an exponential increase in CO_2 emissions and temperature. To understand the impact of climate change and potential risks, future scenarios have been developed by the Intergovernmental Panel of Climate Change (IPCC), which are based on diverse degrees of mitigation efforts to decrease CO_2 emissions. The low mitigation scenario SSP5-8.5, predicts that by the end of this century, sea surface temperature (SST) will have increased by

2.58°C, seawater surface pH will have decreased to 7.7; and atmospheric CO_2 could have reached 1000 μatm. In contrast, the high mitigation scenario SSP1-2.6 corresponding mean changes are +0.73°C SST, reduction to pH 8.0 for surface seawater, and approximately 430–480 μatm CO_2 concentrations (IPCC, 2014; Pörtner et al., 2019; IPCC, 2021).

The combined effects of ocean acidification (OA) and warming on marine life have been studied for at least two decades, but they are still challenging to interpret and predict. A growing number of experiments using ecologically and economically important species, such as Pacific herring (Villalobos et al., 2020), Pacific oysters (Lemasson et al., 2018), gilthead seabream, meagre (Pimentel et al., 2016), American and European lobster (Small et al., 2015; Waller et al., 2017) have shown an exacerbated impact of OA on survival, physiology, and growth when it was combined with elevated temperatures. Synergistic impacts (the result of stressors interacting and producing a greater effect than the cumulative or individual effects) of climate change vary across life stages with the tendency that early life stages are more sensitive and less tolerant to environmental stressors than adults (Kikkawa et al., 2003; Ishimatsu et al., 2004; Kurihara, 2008). Understanding the synergistic effects of OA and warming on larval development is critical to predict how climate change will influence larval survival, dispersal and hence, population connectivity (Cowen and Sponaugle, 2009; Giménez et al., 2020). This is particularly important for the future of commercially important and vulnerable species, like crustaceans, which have complex life cycles and undergo distinct ontogenetical changes. As in the majority of marine species with planktonic larvae, the transition between the larval pelagic stage to the benthic post-larval stage larvae has been described as a population bottleneck (Marshall and Morgan, 2011).

European lobster (*Homarus gammarus*) develops through three pelagic larval stages (stages I, II and III), a postlarval stage (stage IV) and then reaches the juvenile stage which marks the complete transition to a benthic lifestyle (Charmantier et al., 1991). The International Union for Conservation of Nature has listed the European lobster as "least concern" as the examination concluded that this species has a broad geographic range, despite commercial fisheries. This is perhaps true on a global level, but not for the lobsters of the German Bight, North Sea, that inhabit the rocky shores of the island of Helgoland. This population experienced a dramatic decline in the 1950s and 1960s from a combination of overfishing, pollution and extensive habitat destruction (Franke and Gutow, 2004). The decline of this population prompted a large-scale restocking program on Helgoland, presently carried out by the lobster conservation company, Reefauna. After 10 years (1999 – 2009) of releasing hatchery-reared juveniles into the wild, the success of the restocking program was evaluated; the results showed that re-stocked lobsters could be re-caught; survival rates averaged 40% and the proportion of caught cultured lobsters to wild lobsters was 3–8% between the years 2007–2009 (Schmalenbach et al., 2011). Nevertheless, despite recapture of marked lobsters, it is unknown whether recruitment is successful. Most of the catches were older individuals and lobster larvae are rarely caught in long-term plankton net monitoring around the island (Greve et al., 2004). Therefore, the question arises if recruitment of remaining natural individuals and program-released specimens is currently successful, or could be affected by ongoing climate change (i.e. ocean warming and acidification).

Only two studies have assessed the joint effects of OA and ocean warming on lobster larvae of the genus *Homarus*. They provide the first insight on how lobsters may respond to the synergistic effects of environmental changes predicted for the end of the 21st century (Small et al., 2015; Waller et al., 2017). These studies have in common an experimental design based on only two temperatures and two pCO_2 regimes, comparing (in a factorial design) ambient temperature and pCO_2 conditions with increased temperature and pCO_2. Both studies demonstrated that elevated temperature has a stronger effect on life history (survival and development) and physiological responses (oxygen consumption rates) of lobster larvae than elevated pCO_2. Nevertheless, it remains unknown how lobsters will react to a broader range of temperatures under ocean acidification. Regional differences from the global mean SST and CO_2 uptake trends can result in a "temperature buffering" effect, possibly mitigating some of the negative impacts of OA. Therefore, as suggested by Humphreys (2017), OA experimental setups should be combined with a thermal gradient to reflect regional variation from the global mean SST more realistically.

How marine crustaceans will perform under future high CO_2 can be interpreted by their physiological capacities to adjust to environmental change. Crustaceans are water breathers and are directly exposed to ocean acidification through their gills which are specialized for respiratory gas and ion exchange (Taylor and Taylor, 1992). An acute rise in seawater pCO_2 reduces (or reverses) the pCO_2 diffusion gradient across the gills, causing additional CO_2 to accumulate in the haemolymph (extracellular compartment) until an excretory gradient is restored (Whiteley, 2011). Regulating haemolymph pH is necessary to maintain proper oxygen supply, when the concentration of CO_2 in the haemolymph increases and pH decreases it causes hemocyanin (oxygen transporting proteins) to release their load of oxygen molecules as explained by the Bohr effect (Hirota et al., 2008; Strobel et al., 2012). Nonetheless, crustaceans are equipped to buffer changes in haemolymph pH to some extent through iono-regulation (Whiteley et al., 1999; Whiteley, 2011). However, acute and long-term exposure to OA could interrupt this acid-base equilibrium and alter metabolism and growth (Whiteley and Taylor, 1992; Whiteley et al., 1999). Thus, routine metabolic rate (RMR) is traditionally investigated in studies on multiple environmental stressors as an approach to assess if elevated CO_2 concentrations affect the sensitivity of organisms to thermal extremes (Storch et al., 2011; Waller et al., 2017; Laubenstein et al., 2019). At optimal temperatures, organisms have maximal aerobic capacity and proper functioning (Pörtner, 2001). While at suboptimal temperatures aerobic capacity is limited and failure to sustain a balance between metabolism, development and growth can result in reduced body mass at critical life history stages (Anger, 2001; Pörtner, 2008; Torres and Giménez, 2020). The suboptimal temperatures can be divided into the *pejus* range,

where an organism performance starts to decrease (Frederich and Pörtner, 2000; Frederich et al., 2009); and the *pessimus* limit, when an organism switches from aerobic into anaerobic metabolism (Jost et al., 2012). Additionally, the energetic costs of maintaining proper functioning under increased pCO_2 levels can further interrupt defense mechanisms against reactive oxygen species (ROS), leading to oxidative stress and lipid peroxidation (Rato et al., 2017). Therefore, the decrease in antioxidant response and consequent lipids' oxidative damage can serve as a proxy to identify when an organism's defense mechanism has been compromised or suppressed by environmental stressors (Beliaeff and Burgeot, 2002; Rato et al., 2017; Tremblay et al., 2020).

The purpose of our study was to provide a more complete picture on how European lobster larvae will perform in future CO_2 conditions by including a wider range of temperature treatments. Our study investigated the ability of European lobster larvae to survive and develop successfully as well as their aerobic metabolic capacity when exposed to the projected SPP1- 2.6 scenario OA conditions and a temperature range that covers cold and warm suboptimal temperatures. Larvae from four independent hatches were reared from stage I to III under a gradient of 10 different seawater temperatures (13–24°C) combined with moderate (average ~470 µatm) and elevated (average ~1160 µatm) seawater pCO_2 treatments (corresponding to the very stringent [SSP1-2.6] and worst-case emission scenario [SSP5-8.5] projected for 2100 by IPCC).

MATERIALS AND METHODS

Animal Collection and Maintenance

The study was carried out at AWI Helmholtz-Zentrum für Polar- und Meeresforschung (Helgoland, Germany). The experiment was repeated four times under the same temperature and light regimes (12:12 h light/dark), each experimental run was carried out with larvae from different females or hatches, hereafter referred to hatches. Hatches typically vary due to genetic or maternal effects. Thus, pooling larvae together from different females could potentially mask responses to the treatments. This is the reason why we chose to repeat the experiment with four independent hatches to increase the robustness of the results. European lobster larvae (*Homarus gammarus)* hatched during summer from four ovigerous female lobsters captured by local fishermen in the rocky subtidal zone around the island of Helgoland (German Bight, North Sea, 54°11:3′N, 7°54.0′E). Females were fed by the lobster conservation company Reefauna and kept in separate tanks (29 × 79 cm), filled with running seawater from the North Sea under a natural light cycle until hatching occurred. Freshly hatched larvae were transferred to 60 × 800 ml glass beakers and were distributed evenly into four sections to minimize cannibalism. Two 100 cm^2 plastic meshes (mesh size: 500 µm) were sewn in the middle and placed in the beaker to delimit these areas. The number of larvae was 15 per beaker for the first hatch and was adjusted to 12 afterwards for better survival. In total 2,880 lobster larvae were used. The research presented in this paper complies with the guidelines from the directives 2010/63/EU of the European parliament and of the Council of 22nd September 2010 and the German law on the protection of animals used for scientific purposes.

Experimental Design and Seawater Parameters

A thermal gradient incubator similar to the one used by Thomas et al. (1963) was used for the experimental setup. The table was built to hold 60 beakers (10 columns × 6 rows) and connected to two cooling bath thermostats with engine coolant flowing through a closed system (HUBER Compatible Control K6, Offenburg, Germany) that were set at 11.8 and 27.8°C. A gradient of 10 temperatures was obtained in the horizontal axis (**Supplementary Table S1**). On the vertical axis, two CO_2 concentrations were set at target 450 µatm (SSP1-2.6 scenario) and 1150 µatm (SSP5-8.5 scenario) and supplied with gentle bubbling in three rows for a total of 30 beakers per CO_2 concentration. Each beaker had a plastic hose with a glass tube extremity connected to a CO_2 distributor. The targeted CO_2 levels were reached using a system that removes CO_2 from ambient air with a soda lime filter. The CO_2-free air (<1 µatm CO_2) was mixed with pure CO_2 (Air Liquide Deutschland ltd., Düsseldorf, Germany), and the pCO_2 of the mixture was continuously monitored with a gas detection unit (GDZ 401, Umsitec, Denkendorf, Germany) that automatically adjusts the CO_2 concentration and flow rates to maintain the target values. All beakers were covered by a clear plastic bag to limit CO_2 outgassing throughout the experiments. The setup resulted in a triplicate per temperature and CO_2 concentration (*see* **Supplementary Table S1**). Lobster larvae were raised from stage I to stage III under the temperature and pCO_2 conditions related to their position in the gradient table. Larvae were fed *ad libitum* (ca. 200–300 *Artemia salina* nauplii) after the daily water change at 9:00.

Seawater parameters were measured daily (n = 1 for each combination of temperature CO_2 concentration), using a pH meter (WTW pH315i, Wilheim, Germany) and pH electrode (WTW SenTix 21 Basis pH-combined electrode, Wilheim, Germany), salinometer (WTW Cond 3110 SET 1, Wilheim, Germany) and salinity sensor (WTW, Conductivity Cells TetraCon), and thermometer (VOLTCRAFT DET2R, Wernberg-Köblitz, Germany) (*see* **Supplementary Table S1**). Total alkalinity (TA) was measured at the beginning and end of all experimental runs (n = 2 for each combination of temperature CO_2 concentration). For TA, water was sampled airtight in 100 ml bottles and stored at 4°C until later measurements with a TitroLine α plus titrator (SI Analytics GmbH [Xylem], Weilheim, Germany) in technical duplicates with Dickson Batch 104 (NOAA, Reference material for oceanic CO_2 measurements, 2010) as a standard. The seawater carbonate system was calculated based on measured TA, temperature, pH, salinity and pressure using the CO_2SYS Excel Macro software (Pierrot et al., 2006). The following calculations were used, Mehrbach et al. (1973) refitted by Dickson and Millero (1987) for the CO_2 constant, total scale (mol/kg-SW) for pH scale, Uppström (1974) for total boron and Dickson (1990) for

$KHSO_4$ to calculate the carbonate system. The obtained values are summarized in **Supplementary Table S2**, the mean values of pCO_2 treatments among all temperatures were 467 ± 19 for the moderate pCO_2 treatment and 1156 ± 27 for the high pCO_2 treatment.

Survival and Development Time

Lobster larvae in each beaker were monitored during the daily water change to record mortality and dead larvae were removed immediately. Cumulative survival was expressed as the percentage of the number of larvae introduced into each beaker at the start of the experimental run. To monitor development, beakers were checked daily at 9:00 for evidence of molting. Larvae were individually observed for stage characteristics, such as the formation of pleopods for stage II and the formation of uropods for stage III. When larvae molted to stage III, they were removed from the beaker for further measurements (*see* next sections). Sampling was divided into three groups once larvae reached stage III: *1*) three larvae from each beaker were sampled for RMR and afterwards frozen for biomass, carbon and nitrogen measurements; *2*) three larvae per beaker were photographed for size and morphology analysis and *3*) three larvae from each beaker were immediately frozen for enzymatic antioxidants analysis. Each experimental run lasted approximately 28 days, to allow all larvae in different temperatures treatments to reach stage III.

Routine Metabolic Rate (RMR) Measurements

RMR was used as a proxy to investigate the effect of elevated pCO_2 and temperature on stage III larvae metabolism. RMR measurements were done under the corresponding experimental temperature and freshly prepared pCO_2 conditioned seawater. To make sure larvae were in a post-absorptive state, larvae were starved for 2 h to allow gastric processing (Kurmaly et al., 1990; McGaw and Curtis, 2013) in 20 ml glass vials implemented with an optically isolated oxygen sensor type PSt5 at its bottom (PreSens, Regensburg, Germany). During this 2 h, vials were covered with a mesh to avoid larvae escape and permit oxygen diffusion in the conditioned seawater. This period also allowed larvae to recover from handling stress. After 2 h, vials were tightly closed with a plastic lid, while submerged in the corresponding conditioned seawater in order to avoid air bubbles and placed on a SDR SensorDish® Reader (PreSens, Regensburg, Germany). This system consists of a 24-channel reader of oxygen luminescence quenching and provides a high-quality measurement without oxygen consumption or gas exchange between the environment and the vial functioning as the incubation chamber. The system was calibrated at each temperature with seawater at 100 and 0% air saturation following the manufacturer's protocol. A 12-well microplate was adapted to the system to measure simultaneously 12 glass vials (20 ml). Vials without larvae ($n = 2$) were used as a control to account for microbial oxygen consumption. Vials and channel readers were placed on a rocking platform shaker (IKA Rocker 2D digital, Staufen, Germany) at 80 revolutions per minute (rpm) to avoid oxygen stratification within the vials during measurement. The vials were incubated in the dark with an opaque black plastic box. The oxygen concentration was recorded every 15 s during 4 h. Oxygen levels during measurement were monitored closely to avoid suboptimal levels (<4 mg•L^{-1}) inside the chambers. Oxygen consumption was determined by a linear regression of the change in O_2 concentration data plotted against time. After RMR was measured larvae were frozen for further biomass, carbon and nitrogen measurements (*see* next section) to express RMR in O_2 mg•h^{-1}•mg DM^{-1}. RMR was measured in postmolt larvae to allow comparison at all temperature treatments, as the intermolt period of larvae is greatly dependent on temperature and is thus highly variable. Past studies measuring RMR in lobster larvae show respiration rates are fairly consistent between intermolt and postmolt stage III larvae (Sasaki et al., 2011).

Biomass, Carbon and Nitrogen Content

Freshly molted stage III larvae used for RMR were sampled for dry body mass, and carbon/nitrogen content measurements. Carbon was measured as a proxy for reserves (lipid content) and nitrogen as a proxy for protein content. The same parameters were measured in freshly hatched larvae (8–15 replicates per hatch) (*see* **Supplementary Table S3**). Larvae were rinsed gently with distilled water, blotted dry to remove salts and excess water and stored in 1.5 ml microcentrifuge tubes at −20°C for later analysis. For the analysis, larvae were placed in pre-weighed zinc cartridges (8 × 11 mm, LabNeed, Germany), then freeze-dried for 48 h (Christ Alpha 1–4 freeze dryer, Germany) and afterwards weighed to the nearest 0.0001-mg using a microbalance (Sartorius SC2, Germany). Carbon and nitrogen contents were then measured using an element analyzer (vario MICRO cube CHNS analyzer, Elementar Analysensysteme, Germany).

Dry mass (DM) was measured in freshly hatched larvae (8–15 replicates per hatch) to calculate instantaneous growth. Instantaneous growth rate was calculated as:

$$g = \log\left(\frac{DM_f}{DM_0}\right)\Big/T$$

In this formula DM_f is the corresponding dry mass value at stage III, DM_0 is the dry mass value at hatching, and T is the development time from hatching to stage III. Total production was calculated as an additional parameter to investigate fitness of larvae and was calculated as the number of survivors to stage III in each treatment multiplied by the corresponding dry mass.

Morphological Measurements

As a proxy to assess possible malformation under high pCO_2, as seen in a study on the effect of OA on lobsters (Agnalt et al., 2013), we measured eight morphological traits. Stage III larvae were placed laterally in a Petri dish and photographed using an Olympus SZX16 stereo microscope. Pictures were then analyzed using ImageJ Software (ImageJ 1.45s, National Institute of Health, Madison, WI, United States). Eight morphological characteristics were measured following the protocol of a similar study in American lobsters *(Homarus americanus)* (Menu-Courey et al.,

2019): *1*) rostrum length (RL), *2*) carapace length (CL), *3*) total length (TL), *4*) telson length, *5*) the dominant claw pollex, *6*) the dactylus, *7*) the eye diameter, which consisted of measuring the dark area. The abdomen length was calculated as the difference between TL and the sum of RL and CL.

Antioxidant Enzyme Activity

Stage III larvae were sampled and immediately snap-frozen in liquid N_2 and kept at −80°C until analyzed. To determine the level of cellular stress larvae experienced under experimental conditions and the mechanisms involved in the response, four antioxidant enzymes were analyzed in technical triplicates: superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx) and catalase (CAT). Each individual was cut into two pieces below the carapace and ground in liquid N_2 using a ceramic pestle. The front part (carapace) was used for antioxidant enzymes. We aimed to quantify lipid damage using the abdomen part of the larva *via* malondialdehyde (MDA) formation, but these data were discarded as they were mostly under the detection level. For the enzymes' analysis, the samples were transferred to microcentrifuge tubes with 125 µl of phosphate buffer solution [50 mM potassium phosphate dibasic and monobasic mixture (K_2HPO_4/KH_2PO_4, 30.5 and 19.5% respectively), 50 mM Ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulphonyl fluoride, pH 7.5], homogenised using a laboratory ball mill (MIXER MILL MM 400, Retsch, Haan, Germany) and centrifuged at 23,897 *g* for 3 min at 4°C to obtain the supernatant used for the assays. SOD catalyses the conversion of $O_2\bullet^-$ to H_2O_2 and was measured using xanthine-xanthine oxidase as a superoxide radical generating system and nitroblue tetrazolium as a detector (Suzuki et al., 2000). GST modifies xenobiotics into other conjugates using reduced glutathione (GSH) as substrate, and was estimated by detecting the formation of the thioether product from the reaction between GSH and 1-chloro-2,4-dinitrobenzene (Habig and Jakoby, 1981). GPx removes H_2O_2 using nicotinamide adenine dinucleotide phosphate (NADPH) as substrate and was measured by monitoring the decrease in the concentration of NADPH at 340 nm upon addition of H_2O_2 to the assay mixture (Ahmad and Pardini, 1988). CAT eliminates H_2O_2 too and prevents its accumulation in cells and tissues. The decrease of the H_2O_2 concentration catalyzed by CAT was measured at 240 nm according to Aebi, (1984). Soluble protein was also measured as per Bradford (1976) in all supernatants to obtain enzyme activities expressed in activity units (U)•mg protein^{-1}. All spectrophotometric measurements were done at room temperature (20°C) using a spectrophotometer (THERMO Multiskan Spectrum, Waltham, United States).

Data Analysis

After data visualization, statistical analyses of the defined variables were performed in RStudio Team (2021). Generalized additive models (GAM) with random effects using the package *mgcv* (Wood, 2017) were done with temperature and CO_2 concentration as fixed factors, plus the addition of the hatch as a random factor (specified as: *s(hatch, bs= "re")*) for all measured variables: survival, development time, morphological measurements, RMR, biomass, and antioxidant enzyme activities. The best model (interactive, additive, temperature and CO_2 only, null model) was then chosen based on the Akaike information criterion (AIC) score and simplicity of the model (**Supplementary Table S4**). Line graphs were plotted using the smoothing command from the package *mgcv* (Wood, 2017) with the predicted regression line in *ggplot2 (*Wickham, 2016*)*. The lines are the predicted regression lines: solid black lines were plotted when there was a temperature effect, but no effects of OA. Red and blue lines were plotted when there was a temperature and CO_2 additive effect; the red and blue dots represent each sampled larva under high or moderate pCO_2 conditions respectively. Additionally, a multivariate analysis using a principal component analysis (PCoA) was used to visualize morphological measurements by pCO_2 and temperature treatments and permutational multivariate analysis of variance (PERMANOVA) to test significance.

RESULTS

We did not observe any evidence of a synergistic effect of high temperature and high pCO_2 in any of the studied variables. Statistically this means that in no case the model including interactions between temperature and pCO_2 concentration provided a better fit to the data than the models with the two main factors alone. In general, most variables were affected by temperature and effects of pCO_2 (when present) were additive with respect to temperature. We present our results as the average response of larvae under each experimental condition (pCO_2 and temperature) and hatch for better visualization. For results separated by hatch *see* **Supplementary Figures S1–S5**.

Survival and Development Time

We observed evidence of a negative effect of high pCO_2 on larval survival (**Supplementary Table S4**) but not on development time to reach stage III. At higher temperatures, the differences in average survival between CO_2 treatments were small compared to lower temperatures. However, the best model did not retain a term indicating that smooths are conditional on the CO_2 level. Overall, mean survival for all temperatures under moderate pCO_2 was 33.3% in comparison to 27.9% for high pCO_2. Moreover, survival increased with temperature while duration of development time to reach stage III decreased (**Figure 1**).

Biomass and Carbon and Nitrogen Content

Best models retained temperature but not pCO_2 as predictors (**Figure 2** and **Supplementary Table S4**). Dry mass, carbon and nitrogen content, and C:N ratio increased with temperature. Instantaneous growth also increased with temperature but there was no evidence of an effect of pCO_2 (**Supplementary Figure S6**). Temperature and pCO_2 had an additive effect on total production (**Figure 3**). This result matches the trend and significance seen in the survival results.

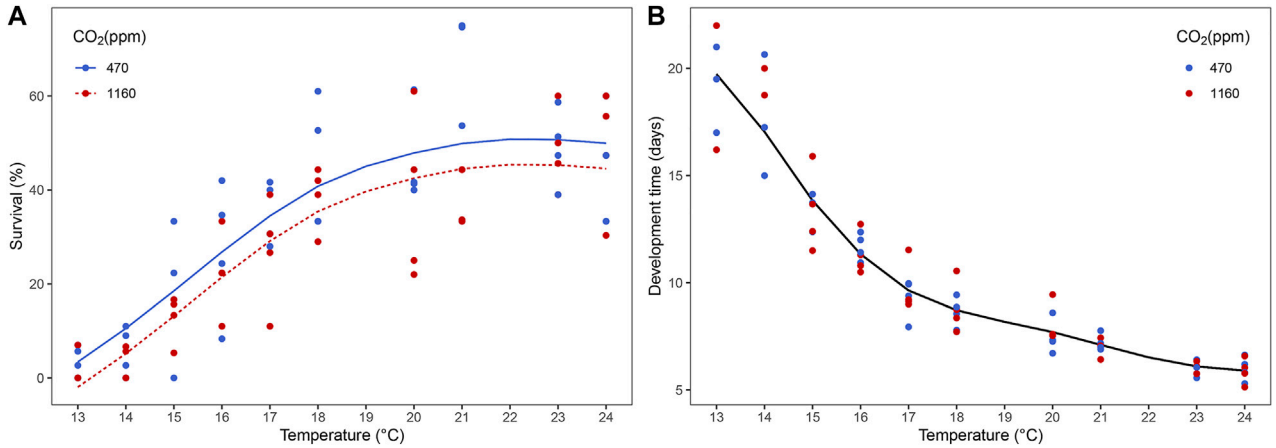

FIGURE 1 | Effect of temperature and ocean acidification on the survival and development time of *Homarus gammarus* larvae to stage III. **(A)** survival was influenced by CO_2 and temperature, **(B)** development time was influenced by temperature. Each point represents the average response quantified in larvae originated from the same female. Curves correspond to smooths fitted with the best (general additive) model, obtained after backwards model selection.

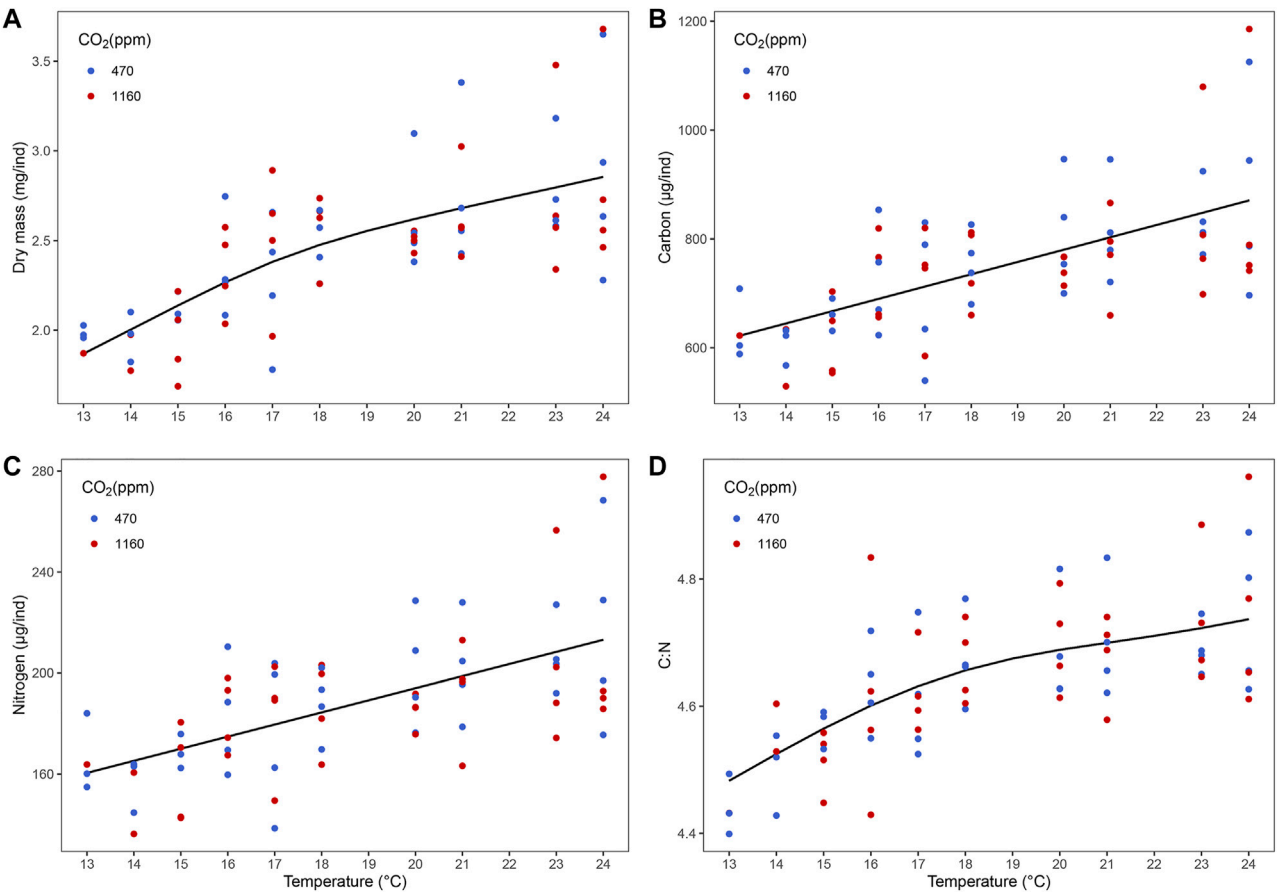

FIGURE 2 | Effect of temperature on biomass of stage III *Homarus gammarus* larvae. **(A)** dry mass, **(B)** carbon content, **(C)** nitrogen content and **(D)** C:N was positively correlated with temperature. Each point represents the average response quantified in larvae originated from the same female. Curves correspond to smooths fitted with the best (general additive) model, obtained after backwards model selection.

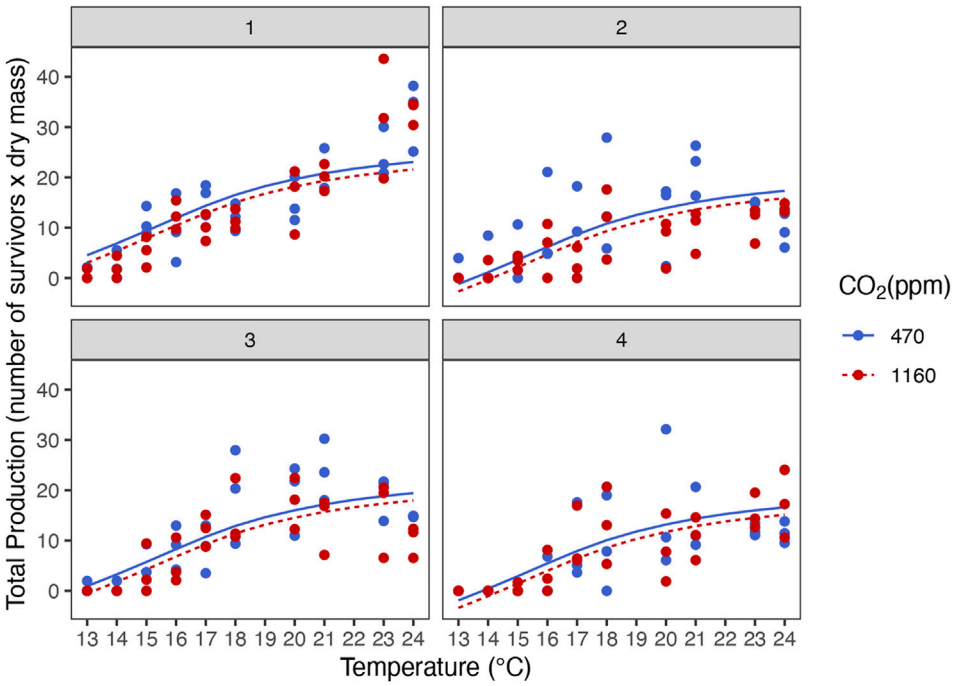

FIGURE 3 | Additive effect of temperature and ocean acidification on total production of stage III *Homarus gammarus* larvae for the four hatches analyzed. Panels **1**–**4** respectively.

Morphological Measurements

Best models retained both pCO_2 and temperature for predictors of rostrum length (**Figure 4** and **Supplementary Table S4**). Lobster larvae in high pCO_2 treatment had, on average, shorter rostrum length than those in moderate pCO_2. Rostrum length (RL) increased with increasing temperature in both CO_2 treatments. For the remaining variables, only temperature was retained in the best model (**Supplementary Table S4**). Carapace length (CL), abdomen length (AL), total length (TL) and claw size increased with temperature (**Figure 4** and **Supplementary Table S4**). By contrast, the CL:AL ratio decreased with temperature (**Figure 4** and **Supplementary Table S3**). Neither temperature nor *p*CO2 were retained as predictors for eye diameter size and telson length. Multivariate analysis using measured morphological characteristics (RL, CL, AL, TL and telson) did not give any significant morphological difference between larvae under moderate and high pCO_2. Temperature had a significant effect on larval morphology in the colder temperatures (**Figure 5**; PERMANOVA test: $F_{1,\ 165} = 7.37$, $p = 0.003$).

RMR Measurements and Antioxidant Enzyme Activity

The routine metabolic rate (RMR) increased with temperature (**Figure 6** and **Supplementary Table S4**). However, we did not find any evidence of an effect of OA (i.e. the best model contained only temperature as predictor). Best models did not retain temperature nor CO_2 as predictors for variation in antioxidant activity of the enzymes SOD, GST, GPx and CAT (**Figure 7**).

DISCUSSION

Contrary to our expectations, we did not find any evidence of synergistic or interactive effects of temperature and pCO_2 on any of the studied response variables. Ocean warming and acidification impacts can range from the highest level of sensitivity seen in the whole organism functioning, down to the cellular and molecular levels (Pörtner, 2008). We accordingly discuss our results from whole body functioning (i.e. survival, growth), to energy metabolism and finally to antioxidant responses. Our findings demonstrate that future high CO_2 concentrations could have an impact on survival and morphology of lobster larvae. However, at the organizational levels analysed here, we did not detect physiological responses in lobster larvae. The latter will be discussed below.

Survival

Our results suggest that near-future pCO_2 conditions have a negative effect on lobster larvae survival to stage III. In lobster larvae, a bottleneck is expected in the transition between the last pelagic stage (stage III) and the benthic (stage IV); the stage III of *H. gammarus* is the one that accumulates most of the body mass leading to the stage IV (Torres et al., 2021). Such bottlenecks are important as life history transitions (Giménez, 2004; Pechenik, 2006; Marshall and Morgan, 2011). Our study clearly showed OA has the potential to increase mortality before reaching the transitional metamorphic stage (stage III to IV). In the wild, this could translate to an additional

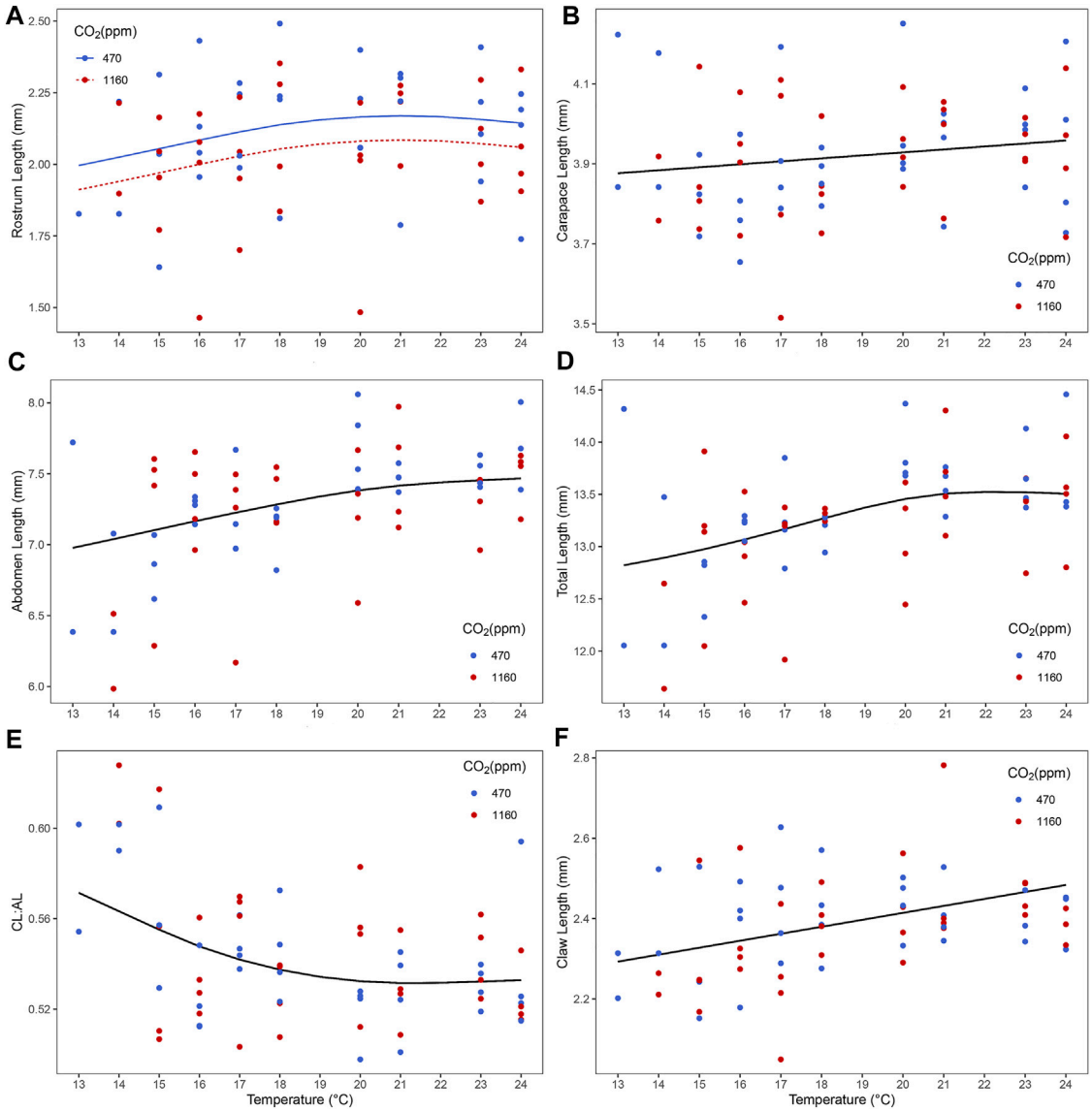

FIGURE 4 | Effect of temperature and ocean acidification on size and morphology of stage III *Homarus gammarus* larvae. **(A)** ocean acidification and temperature effect on rostrum length; and temperature effect on **(B)** carapace length [CL], **(C)** abdomen length [AL], **(D)** total length, **(E)** CL: AL ratio and **(F)** claw length. Each point represents the average response quantified in larvae originated from the same female. Curves correspond to smooths fitted with the best (general additive) model, obtained after backwards model selection.

obstacle for successful recruitment. Similarly, reduced survival due to elevated pCO_2 has been observed in early life stages in the European lobster (Small et al., 2016) and in the congeneric American lobster (Menu-Courey et al., 2019; Noisette et al., 2021). Moreover, this increase in mortality in early life stages of crustaceans exposed to ocean acidification has been noted in several other species: e.g. red king crab, *Paralithodes camtschaticus* (Long et al., 2013), edible crab, *Cancer pagurus* (Metzger et al., 2007) and the porcelain crab, *Pethrolisthes cinctipes* (Ceballos-Osuna et al., 2013). Previous studies on crustaceans and thermal stress revealed elevated pCO_2 can narrow the thermal tolerance of the edible crab, *C. pagurus* and the spider crab, *H. araneus* (Metzger et al., 2007; Walther et al., 2009; Whiteley, 2011). Our survival results provide no evidence of larvae reaching a temperature threshold or *pessimus* range on the warm side of our gradient with a maximum temperature (24°C) under high pCO_2. However, on the cold side, the low number of lobster larvae that reached stage III at 13 and 14°C in both moderate and high pCO_2 treatments suggest the *pessimus* survival limit is below 15°C. The lack of an interaction shows though that the limits were not affected by OA, in contrast to previous observations (stated above) and Pörtner's (2008) predictions. The other significant driver for survival was temperature: higher temperatures resulted in

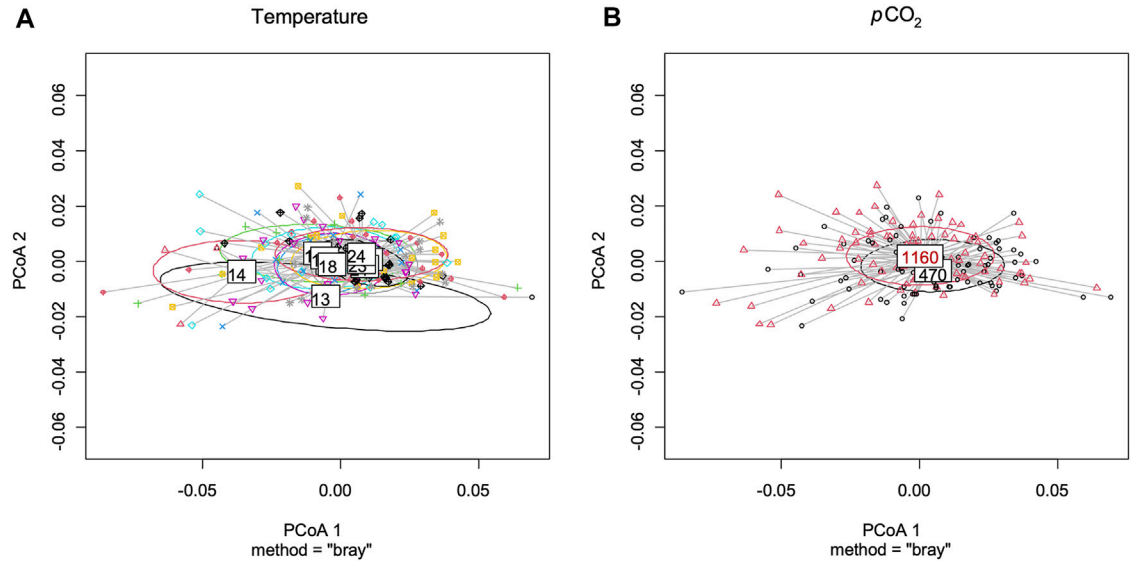

FIGURE 5 | Results of principal coordinates analyses (PCoA) for morphological traits of stage III *Homarus gammarus* larvae. Plots were made using rostrum length, carapace length, abdomen length, total length and telson size. **(A)** comparison by temperatures (13–24°C), significant differences among temperatures. (PERMANOVA test: F1, 165 = 7.37, p = 0.003). **(B)** comparison by pCO_2 concentrations, no significance differences among pCO_2 concentrations.

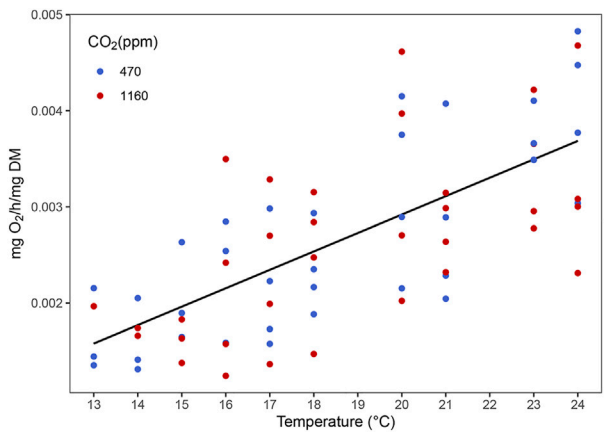

FIGURE 6 | Effect of temperature on the routine metabolic rate of stage III *Homarus gammarus* larvae. Each point represents the average response quantified in larvae originated from the same female. Curves correspond to smooths fitted with the best (general additive) model, obtained after backwards model selection.

higher survival in both moderate and high pCO_2 treatments. We observed similarities with a study carried out in the same region (Helgoland) on the effect of climate warming on European lobster larvae (Schmalenbach and Franke 2010). Their results showed that optimal larval survival occurred within 16–22°C which largely matches our results; we also tested warmer temperatures (23°C and 24°C) where survival was even higher.

Growth: Development Time, Biomass, Carbon and Nitrogen Content

There was no evidence of an effect of elevated pCO_2 on development time from hatching to stage III. Our results are consistent with studies focusing on temperature only (Schmalenbach and Franke, 2010) as well as pCO_2 and temperature (Arnold et al., 2009; Small et al., 2015; Waller et al., 2017) where pCO_2 had no effect on lobster larval development rate. This led us to further enquire if there was possibly a trade-off between slower development rate under pCO_2. For instance, at moderately low salinities, larval development of *H. gammarus* is extended, possibly as a way to minimize the negative effects on lipid and protein levels (Torres et al., 2021). Studies on the combined effect of food limitation and increased temperatures have analyzed the integrated response of dry mass and development under different temperatures (Torres and Giménez, 2020; Griffith et al., 2021) to provide insight if delayed development time could be a compensatory response to maintain body mass (and reserves) at stage. We investigated this integrated response, and our results show there was no trade-off between developing slower under elevated pCO_2 conditions as larvae were reaching similar biomass when molting to stage III (**Figure 8**). Temperature alone was the principal driver in development rate, lobster larvae in warmer temperatures molted to stage III faster independently of pCO_2 treatment. We did not find any evidence of effects of pCO_2 on dry mass and elemental carbon (C) and (N) content, either; thus larvae grew to the thermal-dependent maximum body mass without any need of extending development. If present, the compensatory responses to increased pCO_2 levels operated at a different level of

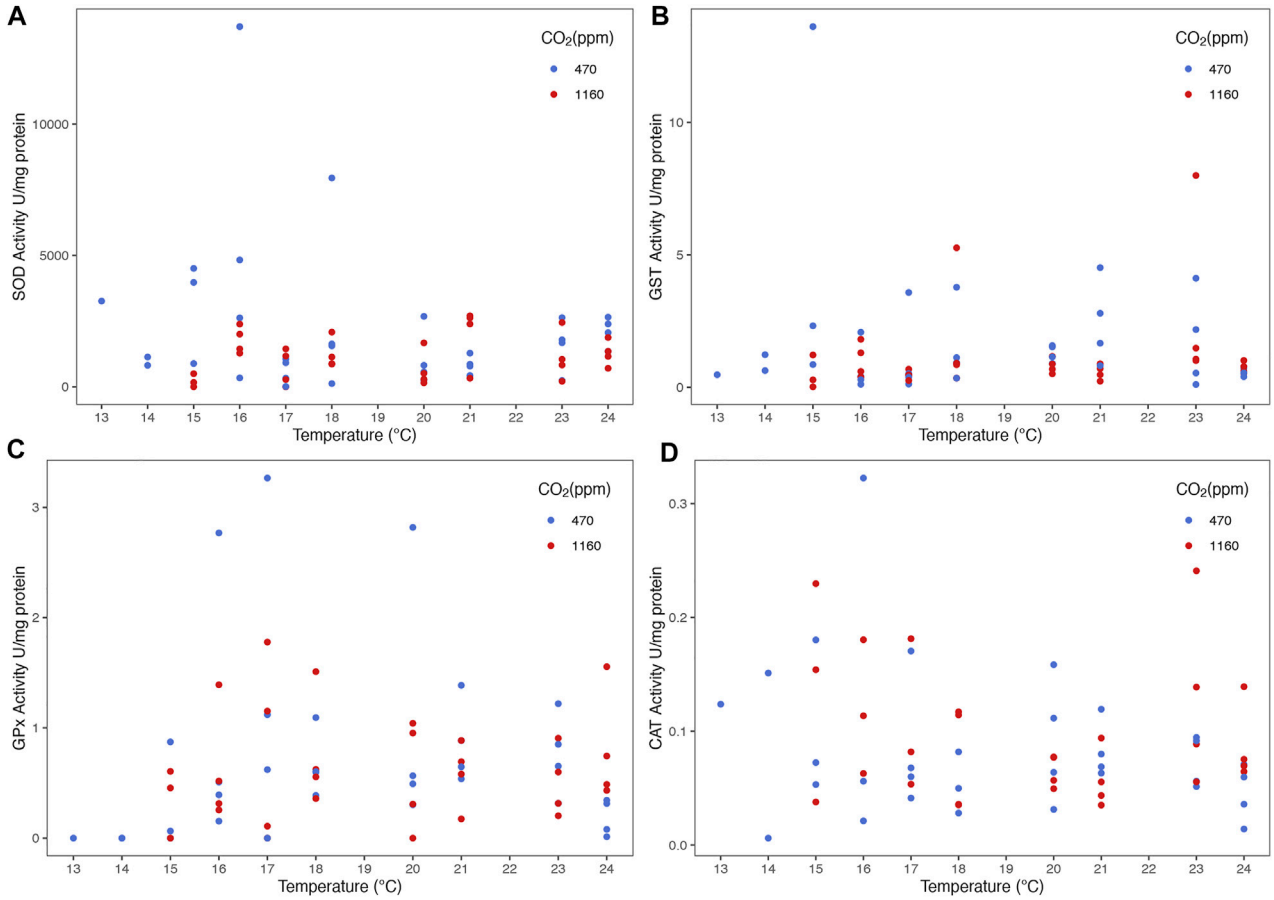

FIGURE 7 | Effect of temperature and ocean acidification on antioxidant enzyme activities of stage III *Homarus gammarus* larvae. There was no effect of experimental conditions (temperature gradient and pCO_2 levels) on antioxidant response: **(A)** SOD, **(B)** GST, **(C)** GPx and **(D)** CAT. Each point represents the average response quantified in larvae originated from the same female. Curves correspond to smooths fitted with the best (general additive) model, obtained after backwards model selection.

organization, potentially at the intracellular level through acid-base balance mechanisms (Whiteley, 2011; Whiteley et al., 2018).

There was an increase in biomass and C and N content with increasing temperature, consistent with the increased survival. In crustacean larvae, carbon content (approximately >35% of biomass) is correlated with lipid content (a proxy for accumulation of reserves), while nitrogen (approximately 8–11% of biomass) reflects the protein content (Anger and Harms, 1990; Anger, 2001; Torres and Giménez, 2020). Biomass can also be divided into composition of proteins, lipids, chitins and free carbohydrates, making up >30%, <20%, <15%, and <5% respectively (Anger and Harms, 1990; Anger, 1998). However, chitin is mainly associated with cuticle formation and plays a minor role in the accumulation and utilization of energy reserves. Likewise, carbohydrates are stored as glycogen and for the synthesis of non-essential amino acids and thus have no significant influence on the C:N mass quotient (Anger and Harms, 1990).

The effect of temperature on biochemical composition in crustaceans has been studied in both field and controlled laboratory conditions. Field experiments showed that temperature changes, related to seasonal variation, can influence biochemical composition of decapod crustaceans (Buckup et al., 2008; Urzúa and Anger, 2013). Whereas laboratory experiments demonstrated that an increase in temperature led to an augmentation in lipid content in adult male whiteleg shrimps *(Litopenaeus vannamei)* (Perez-Velazquez et al., 2003) and a decrease in protein content in the northern shrimp larvae (*Pandalus borealis)* (Brillon et al., 2005). Our results are in line with previous results reporting higher lipid content in cherry shrimp (*Neocaridina heteropoda heteropoda)* at 24°C (Tropea et al., 2015). However, the same authors noted a decrease in lipid concentrations at 28°C and 32°C, which we did not see with our experimental design. We did not explore extremely high temperature where consumption rates of lipids (reflected in a decrease in carbon content) could have increased due to increased energy demands. More specifically, studies on American and European lobsters have shown varying results that are challenging to compare due to the difference in temperature treatments and life stages. A study done by Small et al. (2016) on juvenile European lobster acclimated at 10°C and 13°C showed carbon content decreased and nitrogen increased in the warmer

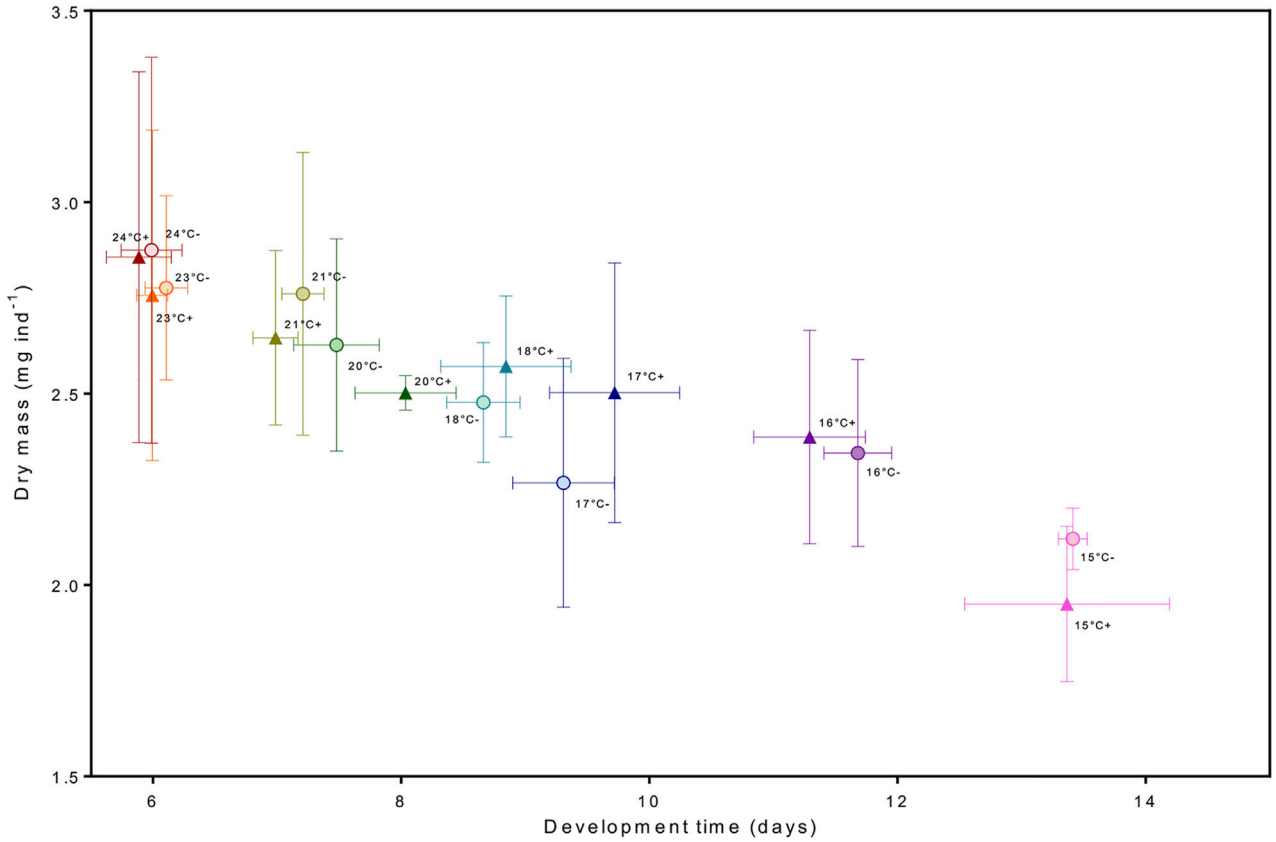

FIGURE 8 | Integrated response between development time and drymass. The circle and (–) sign represent the moderate pCO_2 treatment and the triangle and (+) sign the high pCO_2 treatment.

temperature treatment. Another experiment done on stage I to stage IV European lobster larvae reared at 17°C and 21°C found no significant effects of elevated temperature on organic content (Small et al., 2015). Additionally, an experiment done on OA and warming on American lobster stage III larvae (Waller et al., 2017) showed higher C:N ratio at 19°C compared to 16°C. However, this difference was not seen in stage I, II or IV larvae. Our experiment similarly saw an increase in C:N ratio in stage III larvae at higher temperatures.

The high dry mass, and C and N content matches the energetic demands for rapid growth, accelerated cellular mechanisms and bigger size in warm temperatures. Because C:N ratios were lower towards the lower end of the temperature range, we hypothesize that such low temperatures limited the rate of accumulation of lipids more than that of proteins. Lower changes in proteins than in lipids has also been found when larvae are exposed to low salinity (Torres et al., 2002, 2021). In contrast, on the warmer side an increase in C:N ratio can be indicative of protein degradation due to high metabolism (Weiss et al., 2009). Thus, the decrease seen in C could be related to a degradation of lipids due to extra energetic requirements and a decrease in N could translate to a shortage in protein (Anger and Harms, 1990).

Size and Morphology

Evidence of an effect of high pCO_2 on size and morphology was found only for rostrum size, larvae exposed to high pCO2 showing shorter rostrum length than those in the high pCO_2 treatment. The rostrum is the region which protects the eyes (Ingle, 1997), a reduced rostrum could potentially lead to eye damage; and proper eye-functioning is necessary for localizing prey and predators (Wahle, 1992). Consequently, we measured the eye diameter, as a proxy to assess if there was any correlation with nervous system underdevelopment (Letourneau, 1976; Laverack, 1988; Beltz and Sandeman, 2003) in elevated pCO_2. Nevertheless, despite larvae having smaller rostrum in the high pCO_2 there is no evidence that the eye size was influenced by high pCO2 or high temperatures. Additionally, we checked for "puffy" carapace, bent rostrum, and telson deformities, as seen in past studies of juvenile *H. gammarus* exposed to increased pCO_2 at cold (12°C) and optimal temperatures (18°C) (Agnalt et al., 2013). Our results show only one case of a puffy carapace in larva under high pCO_2 at 24°C. Lobster larvae reduced size under high pCO_2 has been observed before (e.g. in carapace length (Keppel et al., 2012; Rato et al., 2017). Conversely, a study on *H. americanus* found a positive correlation between elevated pCO_2 and carapace and abdomen length (Menu-Courey et al., 2019). We believe the effects on size observed in our study are not as strong as

the study by Menu-Courey et al. (2019) and Noisette et al. (2021) possibly because the pCO_2 gradient treatments used in those studies reached higher concentrations (1200, 2000 and 3000 μatm) than ours. Furthermore, our study only focused on larval stages and decapod larvae exoskeletons are unmineralized while those of benthic juveniles are partially calcified (Anger, 2001). This lack of calcification may be the reason why elevated pCO_2 did not have a stronger impact on the size of the larvae's different body parts.

RMR and Antioxidant Enzyme Activity

Temperature is one of the most important factors influencing routine metabolic rates (RMR) in lobsters and other decapods (McLeese, 1964). Our results show that lobster larvae RMR increased linearly with increasing temperature, independent of pCO_2 treatment. Our findings do not show an exponential relationship between RMR and temperature because this pattern is typically seen in standard and maximal metabolic rate measurements. Moreover, the results are highly dependent on the acclimation of animals, the larvae in our experiments were acclimated to a certain temperature and then respiration rate was measured at the same temperature. Exponential increase of oxygen consumption with temperature is usually seen and obtained with a different methodology (Schulte et al., 2011). A different approach where RMR is measured at acute temperatures independent of the temperature treatment larvae were acclimated to, could have help identify bottlenecks in cell functionality derived from the compensation costs provoked by combined higher temperature and pCO_2 treatments. However, the design of our experiment and sample size did not allow for this kind of approach. The "Temperature Induced Metabolic Rate" method could be tested on lobster larvae in the future, as it is suitable for studying the effects of temperature on the metabolic capacities of non-constantly swimming organisms (Paschke et al., 2018). For this standardized method, the researcher is required to evaluate critical thermal maximum (CT max) and critical thermal minimum (CT min) to set the measurement temperature for high and low metabolic rates at each acclimation temperature to calculate an aerobic budget.

The higher RMR at warmer temperatures can be associated with faster development rate and larger size. In physiology, the cost of growing faster comes at the expense of an increase in feeding rates and thus swimming to catch food. These activities have been suggested to be energetically expensive in planktonic crustaceans (Morris et al., 1985). Our results are in line with previous studies on early life stages of *Homarus sp.* (Small et al., 2015; Waller et al., 2017; Menu-Courey et al., 2019), northern shrimps, *Pandalus borealis* (Arnberg et al., 2013), and juvenile porcelain crabs, *P. cinctipides* (Carter et al., 2013), where pCO_2 did not significantly affect respiration rates. Significant oxidative stress responses would allow us to infer with more certainty on the optimal, *pejus* and p*essimus* ranges of lobster larvae. However, without evidence of significant antioxidant response fluctuations, it is difficult to separate *pejus* and *pessimus* ranges. From the oxygen consumption point of view and survival alone, the optimal range would be temperatures between 17–24°C as lobster were able to use their energy supply to maintain maximal physiological functions. Helgoland's European lobster larvae appear to be quite tolerant to temperatures above those found in the German Bight (Schmalenbach and Franke, 2010).

On the cold side of our temperature gradients, our results show suboptimal temperatures under 15°C, expressed in low survival rates and low RMR. These temperatures are unusual for summertime in Helgoland when lobster larvae hatch (Schmalenbach and Franke, 2010). Nevertheless, temperatures recorded at the Helgoland long-term sampling indicate temperature increases are most noticeable during winter (Franke et al., 1999; Wiltshire et al., 2008). Experimental evidence shows winter warming (+3°C) can alter larval recruitment and result in lobster larvae hatching earlier, mid-April instead of mid-June. In the wild, lobster larvae could be faced with suboptimal temperatures that could cause the lengthening of development time in the pelagic stage, thus increasing the danger of mortality through predation (Schmalenbach and Franke, 2010).

Enzymatic antioxidant responses were measured for the first time in lobster larvae exposed to multiple environmental stressors. To date there is only one study by Rato et al. (2017) that analyzed the biochemical responses of *H. gammarus* under acidification alone and highlights the occurrence of oxidative stress. They found out lobster larvae under high pCO_2 (710 μatm) had reduced SOD and higher DNA damage. Our study included variables not measured before under OA and thermal stress, such as the enzyme activity of GST, GPx and CAT. However, there was no evidence that OA and temperature had a negative impact on the antioxidant enzyme activity. We recognize deeper investigation at the molecular level (proteomic or transcriptomic) could reveal further information on the processes lobster larvae go through to cope with elevated pCO_2 (Noisette et al., 2021). For instance, Noisette et al. (2021) findings show elevated pCO_2 (up to 3000 μatm) did not have an effect on larvae at a physiological level, however, there is evidence they underwent intensive metabolic reprogramming.

In conclusion, European lobster larvae demonstrated to be resilient to near future pCO_2 concentrations at temperatures beyond 17–18°C, including higher temperatures than those experienced by the local population. Our results show larvae do not appear to have reached the critical temperatures or *pejus* range under the elevated temperatures tested (23–24°C). Raising the temperature even further and reaching the thermal limit of lobster larvae would have been interesting from a physiological point (e.g. higher antioxidant responses and compromised respiration). However, for the purposes of our research question, we wanted to understand how lobster larvae will cope with the predicted SSP5-8.5 scenario for 2100 in which SST will increase by 2-3°C. We observed no interactive effect of temperature and pCO_2 on the measured variables; temperature was the greatest driver and there was an additive effect of pCO_2 and temperature on survival and size. Examining the results from the perspective of different levels of biological organization, even though pCO_2 did not elicit a response at the cellular level (i.e. enzyme activity) or physiological level (i.e development time to reach stage III); at the population level (survival) there were significant negative effects. We used total production (survival times biomass) as a way to integrate physiological and population

responses, and it was evident there was an increase in mortality in larvae exposed to high pCO_2 accompanied with lower biomass in the suboptimal temperatures (<15°C). Integrating physiological responses to environmental stressors and life history traits is key for species conservation strategies and stock enhancement management. Worst-case climate change scenarios could thus potentially have repercussion on ongoing restock efforts of endangered populations under recovery, like the European lobster population of Helgoland.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MB acquired funding for the experiment. RK provided resources. All authors were involved in experiment design, LL and NT carried out the experiment. LL, NT, and LG analyzed the data. NT, MB, GT, and LG supervised the project. LL wrote the first draft of the manuscript; all authors revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.809929/full#supplementary-material

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CHAPTER 4. NOISY WATERS CAN INFLUENCE YOUNG-OF YEAR LOBSTERS' SUBSTRATE CHOICE AND THEIR ANTIPREDATORY RESPONSES

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Noisy waters can influence young-of-year lobsters' substrate choice and their antipredatory responses☆

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ABSTRACT

Offshore human activities lead to increasing amounts of underwater noise in coastal and shelf environments, which may affect commercially-important benthic invertebrate groups like the re-stocked Helgoland European lobster (*Homarus gammarus*) in the German Bight (North Sea). It is crucial to understand the impact tonal low-frequency noises, like maritime transport and offshore energy operations, may have on substrate choice and lobsters' behavior to assess potential benefits or bottlenecks of new hard-substrate artificial offshore environments that become available. In this study, we investigated the full factorial effect of a tonal low-frequency noise and predator presence on young-of-year (YOY) European lobsters' in a diurnal and nocturnal experiment. Rocks and European oyster shells (*Ostrea edulis*) were offered as substrate to YOY lobsters for 3 h. Video recordings (n = 134) allowed the identification of lobsters' initial substrate choice, diel activity and key behaviors (peeking, shelter construction, exploration and hiding). To ensure independence, YOY lobsters in the intermolt stage were randomly selected and assigned to the experimental tanks and used only once. We provide the first evidence that stressors alone, and in combination, constrain YOY lobsters' initial substrate choice towards rocks. During nighttime, the joint effect of exposure to a constant low-frequency noise and predator presence decreased antipredator behavior (i.e., hiding) and increased exploration behavior. Noise may thus interfere with YOY lobsters' attention and decision-making processes. This outcome pinpoints that added tonal low-frequency noise in the environment have the potential to influence the behavior of early-life stages of European lobsters under predator pressure and highlights the importance of including key benthic invertebrates' community relationships in anthropogenic noise risk assessments. Among others, effects of noise must be taken into consideration in plans involving the multi-use of any offshore area for decapods' stock enhancement, aquaculture, and temporary no-take zones.

1. Introduction

Sounds generated by human activities can be divided into high-intensity impulsive noise (e.g. pile-driving, underwater blasting) and low-frequency stationary noise (e.g. tidal and wind turbines) (Peng et al., 2015; Tidau and Briffa, 2016; Duarte et al., 2021). High-intensity impulsive noise has been linked to numerous negative responses amongst marine mammals (Lucke et al., 2009), fish (Casper et al., 2013, Fewtrell and McCauley, 2012) and invertebrates (Day et al., 2019). In turn, constant exposure to low-frequency stationary noise has received less attention, but hearing sensitivity curves for decapod crustaceans such as common prawns (*Palaemon serratus)*, mud crabs *(Paneopus* spp*)* and Norway lobsters (*Nephrops norvegicus)* show the highest sensitivity for the lowest tested frequencies (100 Hz, 75 Hz and 20–200 Hz respectively) (Goodall et al., 1990; Hughes et al., 2014; Lovell et al., 2005). Crustaceans are more perceptive to low-frequency particle motion instead of sound pressure variations due to their lack of gas filled organs (e.g. swim bladders) (Breithaupt, 2002). Particle motion

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detection is used by invertebrates to locate sound sources in their environment (Popper et al., 2014; Hawkins et al., 2015; Nedelec et al., 2016). Locating the position of a sound source can allow animals to determine the direction and distance of predators or prey and consequently respond appropriately to the stimuli (Popper and Hawkins, 2018). Experimental results suggest that low-frequency (generally defined as <500 Hz) (Carey and Evans, 2011) anthropogenic noise can alter several key behaviors in crustaceans such as: foraging, antipredator responses, shell searching behavior and grouping behavior (Wale et al., 2013a; Roberts and Laidre, 2019; Tidau and Briffa, 2019a). Furthermore, Filiciotto et al. (2014) observed that boat noise induced biochemical stress responses in Mediterranean spiny lobsters (*Palinurus elephas)* and increased locomotor behavior. The latter may be ecologically detrimental as animals may expose themselves more to predators under noisy conditions.

The European lobster (*Homarus gammarus*) habitat extends along the west coast of Europe, from northern Norway to the Iberian Peninsula and further south to the Azores and Morocco. They are also present in most of the Mediterranean Sea (Holthuis, 1991). Young-of-year (YOY) lobsters' density is usually used as recruitment index in lobster populations (Howell, 2012) and to identify nursing grounds, as YOY lobsters have molted to juvenile stages within the past 12 months (Cowan et al., 2001). In the German Bight, the Helgoland European lobster population was an important source of income for the island until the 1950s, with landings reaching 50 T yr^{-1} (Klimpel, 1965). A combination of overfishing, pollution and extensive habitat destruction by the bombing of the island during and after the Second World War, are used to explain the 1960s' collapse in fisheries yields and population densities (Franke and Gutow, 2004). To counter this, the Alfred-Wegener-Institut (AWI) Helmholtz-Zentrum für Polar-und Meeresforschung-Biologische Anstalt Helgoland initiated a program between 1999 and 2009 to investigate whether lobster restocking was possible (Schmalenbach et al., 2011). Through this initiative, a total of over 14,000 YOY lobsters were released between 2000 and 2017 around Helgoland. Presently, restocking has taken off in earnest through the lobster conservation company Reefauna, with up to 6000 YOY lobsters released annually. As the construction of an offshore wind farm cluster in the German Bight (North Sea) led to an examination on the potential of turbine foundations as an artificial hard substrate habitat for decapods (Krone et al., 2017), YOY lobsters were released in these sites in a pilot project. Moreover, this study (Krone et al., 2017) is often cited in Germany and elsewhere in offshore wind farm multi-use project proposals to support the concept of founding structures as suitable rocky habitats for key exploitable species (Tonk and Rozemeijer, 2019). In the North Sea, offshore wind farms, as well as approximately 1500 shipwrecks (Krone and Schröder, 2011), few glacial bolder fields (BSH, 2016) and the natural subtidal cliffs of Helgoland offer substrata for the development of the local population of several species requiring hard substrates, including the European lobster (Schütt et al., 2001).

To tackle the lack of knowledge about the responses of *H. gammarus* to anthropogenic low-frequency sources, which are increasing in its natural environment, we investigated the full factorial effect of low-frequency tonal noise in addition to crab predator presence on hatchery born YOY European lobsters. We used YOY lobsters because this life stage is vulnerable as its survival depends on finding suitable shelter for protection. YOY lobsters are cryptic in the wild as they spend most of their time hiding in complex shelters such as rocks, seaweed, reefs (Able et al., 1988; Lawton and Lavalli, 1995), and other interstitial spaces like cobble and mussel shells (Linnane et al., 2000). As substrates, we used rocks and shells of European oyster (*Ostrea edulis*) because past studies on substrate selection on the congeneric American lobster (*Homarus americanus)* revealed that substrates with preformed crevices were the preferred settlement habitats (Botero and Atema, 1982). The use of oyster shells in particular is motivated by the fact that historically large sections of the German Bight were covered by reefs of *O. edulis*, which may have played an important role in providing refuge to young lobsters. The "RESTORE- Restoration of the stocks of the European oyster in the German North Sea" program, financed by the German Federal Agency for Nature Conservation (BfN) and conducted by AWI, aims to reestablish the extensive sublittoral European oyster reefs that were extirpated by overfishing and demersal trawlers in the 1950s (Gercken and Schmidt, 2014; Pogoda, 2019). As O. edulis can form a reef-type habitat, which offers small crevices for small benthic fauna to seek shelter, assessing the suitability of oyster reefs for released YOY lobsters is a pertinent topic.

We included predator presence as a potential driver of behavior because predatory crabs, like the green crab *Carcinus maenas* (Rayner and McGaw, 2019; Mercer et al., 2001) and the edible crab *Cancer pagurus* (van der Meeren, 2000) are common in the subtidal habitats of the German Bight. Natural and artificial hard-substrate habitats available in the North Sea should provide shelter and resources to juvenile lobsters and potential predators, such as *C. maenas* which can influence foraging and shelter behavior (time spent hiding) of juvenile lobsters (Rossong et al., 2011). Few studies have investigated whether noise is an additional stressor that alone or in combination with predator presence has an influence on substrate choice and behaviors associated with survival. The assessment of prey-predator and substrate choice relationships in a constant low-frequency noise context can provide insights into natural and artificial hard-substrate community dynamic exposed to low-frequency anthropogenic noise.

We aimed at answering three questions: (1) Does tonal low-frequency noise and/or predator presence affect the behavior of YOY lobsters? (2) In the view of European oyster reestablishment, do YOY lobsters prefer to seek shelter amongst oyster shell or rock substrates? (3) Does noise exposure and/or predator presence influence this choice?

2. Material and methods

2.1. Origin of animals and maintenance

The study was carried out at AWI Helmholtz-Zentrum für Polar-und Meeresforschung (Helgoland, Germany). Young-of-year (YOY) European lobsters (*H. gammarus)* were born from ovigerous females captured by local fishermen in the rocky subtidal zone around the island of Helgoland (German Bight, North Sea, 54°11:3′N, 7°54.0′E). After hatching, lobsters were reared in batch cultures until post-larval stage and kept in individual basins connected to a flow-through seawater system (pH: 7.98 ± 0.08, salinity: 33.63 ± 0.22, temperature: 14.78 ± 0.20) under natural photoperiods. Lobsters were fed daily *ad libitum* with *Artemia salina* nauplii. YOY lobsters with carapace length of 13.0 ± 1.5 mm (mean ± SD) were used for the experiments. To act as predator, green crabs (*C. maenas)* with carapace width of 62.0 ± 1.0 mm were captured two weeks prior to the experiments by research vessel *Aade* using baited traps. Crabs were kept in holding tanks in a temperature (14 °C) and light (12:12 h light/dark) controlled room with circulating ultraviolet and biofiltered seawater. Crabs were daily fed with frozen shrimps prior to their transfer to the experimental tanks.

2.2. Experimental design

Experiments were carried out under the following setup in a separated temperature (14 °C) and light (12:12 h light/dark) controlled room. The bottom part of 16 identical plastic rectangular tanks (56.6 × 36.0 × 42.0 cm) filled with 60 L of seawater (pH: 7.97 ± 0.04, salinity: 32.72 ± 0.77, temperature: 14.27 ± 1.00) was uniformly covered with a 3.0 cm layer of coarse sand. The tanks were divided into four equally sized substrate areas providing shelters (each corner; 19.0 × 17.0 cm) containing (1) rocks (6.0–14.5 cm long) placed close together and on top of each other, creating spaces and crevices and (2) *O. edulis* shells (9–12 cm long) likewise placed close together and on top of each other (Fig. S1). Each tank had rocks and oyster shells substrates placed diagonally across each other and their position was alternated for each run.

At the center of the tank, a concrete tetrapod (height: 7.5 cm, width: 9 cm) was attached to a plastic mesh cage to hold the crabs (12 × 6.5 × 5 cm; 5 mm mesh size) and a "noise egg" (see explanations section 2.3; de Jong et al., 2017) was positioned on top. This setup was the same for all tanks independent of treatment in order to maintain an identical landscape. Each run, the tanks were physically placed randomly around the experimental room and then were randomly assigned to one of the four conditions: control (C), predator presence (P), added noise (N) and added noise + predator presence (NP). In the C and P treatments, the vibration-motor inside the noise egg was without batteries, and the crab cage was empty for N and C treatments. Above the tanks, a scaffold with a video camera (MQ8 Mini Camera, Shenzhen Qilesi Electronic Commerce Co, China) was placed to video-record lobster behaviors. This low-cost video camera allows multiple video recording at the same time with high-resolution imaging. The only downside is the limited recording time, which was about 1h30 in most cases. YOY lobsters in the intermolt stage were randomly selected and assigned to the experimental tanks and used only once to ensure experimental independence. All YOY lobsters were fed 3 h before the start of each experimental run. Each run, YOY lobsters were individually removed from the rearing compartment and released into the upper or lower side of the experimental tank equidistant from rocks and oyster shells (Fig. 1). At the moment of release into the experimental tank the corresponding condition (C, N, P, NP) was already in effect. All experimental tanks and substrates were rearranged, rinsed and refilled with new seawater in between trials. The daytime experiments were done in two daily runs from 9:00–12:00 h and 13:00–16:00 h, when YOY lobsters (n = 48; distributed into C = 11, N = 12, P = 11, NP = 14) were video-recorded and assigned to the experimental conditions. The nighttime experiments were done during natural darkness hours to respect the biological clock (18:00–21:00 h). For this setting, the tanks were fitted above with redlights (Mitras GHL, Prolifux) to record the lobsters' behavior without any disturbance. For the nighttime experiment, an increased number of YOY lobsters (n = 86; distributed into C = 23, N = 19, P = 22, NP = 22) were filmed and assigned to the experimental conditions. Daytime and nighttime YOY lobsters were a distinct set of individuals, and each lobster was used only once. As the experiment caused a minimum period of discomforts for YOY lobsters (3 h exposure), all specimens used were later returned to the Reefauna facilities. No post-experimental stress was observed. All procedures presented in this paper comply with the guidelines from the directives 2010/63/EU of the European parliament and of the Council of 22nd September 2010 on the protection of animals used for scientific purposes.

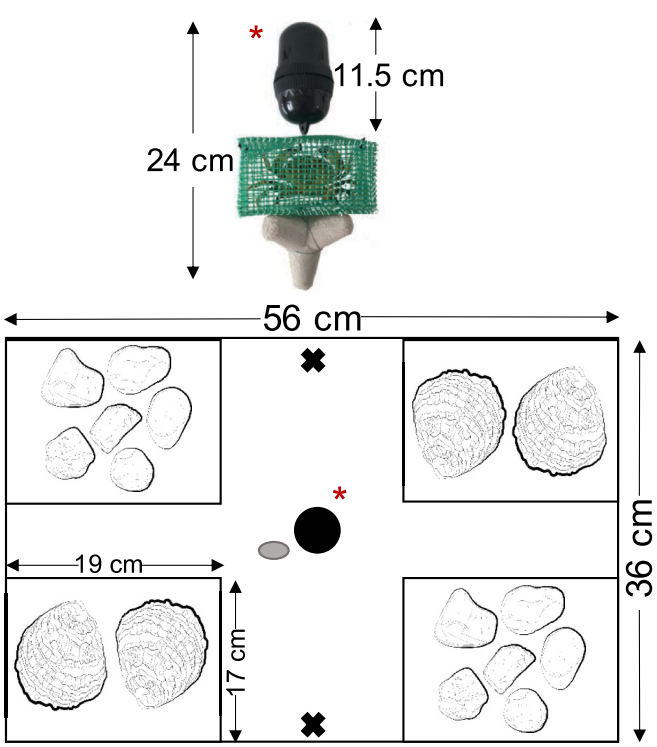

Fig. 1. Diagram of experimental setup. At the center of the tank, a concrete tetrapod was attached to a plastic mesh cage to hold the crabs and a "noise egg" was positioned on top. Top view of experimental tank shows noise egg position (red asterisk), substrates (rocks and oyster shells), two release sites of lobsters (black X), and hydrophone position (gray oval). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Sound characteristics

We used the "noise egg", a standardized device to produce low-frequency underwater noise for laboratory and field experiments. The sound produced is characterized as a constant low-frequency multi-tone with source frequency around 100 Hz (de Jong et al., 2017). The device has been previously used in aquarium experiments on the effect of noise on fish communication and courtship behavior (de Jong et al., 2018a; de Jong et al., 2018b). To confirm lobsters were exposed to distinct sound treatments (ambient and added noise), two calibrated SM3 hydrophones (sensitivity of −165 dB re: 1 V/μPa, Wildlife Acoustics Song Meter Automated Audio Recorder SongMeter, Wildlife Acoustics Inc., USA) were positioned on the sand beside the tetrapod (10.0 cm distance) to record the noise levels in all treatments (Fig. 1) in runs without lobsters during 4 h. The hydrophones were programmed at a sampling rate of 44, 100 (44•1 kHz) samples per second. The sound pressure level (SPL) and the Power Spectral Density (PSD) were computed, calibrated and visualized on 10 s records (see Table S1 for the noise conditions in each experimental treatment) using the R PAMGuide code (Merchant et al., 2015) (SPL: Fast Fourier Transforms 44,100, Hann window, 50% overlap, average for each file; PSD: Fast Fourier Transforms 44,100, Hann window, 50% overlap, 1 s average). For each treatment, recorded sounds were concatenated to form a single contiguous spectrogram. The correction factor for calibration was calculated using manufacturer's technical specifications, the sensitivity of the transducer, the user-defined gain settings for the deployment in decibels, and the zero-to-peak voltage of the analogue-to-digital converter.

We made sure YOY lobsters were exposed to distinct sound treatments by measuring the power spectrum, as in previous studies (Tidau and Briffa, 2019a, 2019b; Hubert et al., 2018). However, a limitation of our study was that we could not model particle motion due to the challenges of working in small tanks and the limitation of not having specialized equipment and sensors to do so (Martin et al., 2016; Roberts et al., 2016; Hawkins and Popper, 2016).

2.4. Video-recording analysis and substrate choice

For the analyses of lobster behavior, we watched 60 min of footage in the daytime experiment due to lower levels of lobster activity compared to nighttime (Fig. S2). The first 5 min were excluded after the lobsters were placed in the tanks to account for tank acclimation. Lobster behaviors that lasted a minimum of 1 s were identified and quantified by a visual continuous sampling procedure (each observed event duration was annotated in a sampling table). The following four behavioral units were defined and observed (Table S1):

Peeking: the lobster rostrum faces toward the outside of the shelter or is visible within the shelter crevices and then withdraws into the shelter in a backward movement or by changing direction; similar to retreat as defined in a previous study (Wickins et al., 1996);

Shelter construction: includes substrate manipulation observed as backward digging (using the first two or three pairs of walking legs and fanning the pleopods) and bulldozing, *i.e.* pushing sand using the chelipeds and first two or three pairs of walking legs (Cobb and Stanley

Cobb, 1971; Berrill, 1974; Wickins et al., 1996);

Exploration: the lobster leaves the substrate completely exposing its whole body (Mehrtens et al., 2005);

Hiding: lobster remains within the substrate and is not visible at all; Substrate choice was assessed manually twice, at the beginning using the video recorded (after 5 min acclimation) and at the end of each trial (after 3 h) when removing the lobsters from the experimental tanks. These were classified as initial and final choice, respectively.

2.5. Data analysis

All statistical data analyses were performed in RStudio (RStudio Team, 2021). For lobster activity analyses, the time spent by each individual engaging in a particular behavior was expressed as a proportion of the total observation time and analysis was carried out in arcsine transformed data. Arcsine transformation was used because data were better fitted when visualized through a quantile-quantile normal plot. Data were analyzed through linear mixed model effects with package *nlme* (Pinheiro et al., 2021). The full model included date of experimental repetition as a random factor, while added noise and predator presence and their interaction were considered fixed factors (full factorial design). Backward model selection for GLM and linear mixed models for both substrate choice and behavior analyses respectively (Table S2 and S3), were carried through the Akaike information criterion (AIC) score. Several models were created starting from the most complex, the full model (interaction between added noise and predator presence), then the additive model (no interaction of factors), then the simplest with added noise and predator presence. Models were then ranked according to their AIC score, when the simplest model had the lowest AIC, that model was chosen. If the difference between the lowest AIC score and the second lowest score (ΔAIC) was greater than 3, the model with lower AIC was selected regardless of differences in complexity. Likelihood ratio test or chi-square test (depending on data set, see Table S2 and S3) was applied as hypothesis testing only when ΔAIC was less than 3, and the most complex test had a lower AIC score. When models differed significantly ($p < 0.05$), the one with lower AIC score was selected. If models did not differ, the simplest model was chosen. Additionally, a multivariate analysis using a principal component analysis (PCoA) to visualize results and permutational multivariate analysis of variance (PERMANOVA) to test significance was performed on all behaviors to complement the univariate analyses (see Fig. S6). For the substrate choice, Holm-Bonferroni was used to test significant difference amongst treatments (pairwise comparison based on p-values from the "summary" function); for time allocation to specific behaviors, Tukey's (HSD) post-hoc comparisons were performed through the package *emmeans* (Lenth, 2021). Substrate choice (*i.e.* the proportion of lobsters choosing a given substrate) was analyzed through generalized linear models (GLM). The full model included time of the day (day or night) and treatment interactions (control, predator presence, added noise and added noise + predator presence).

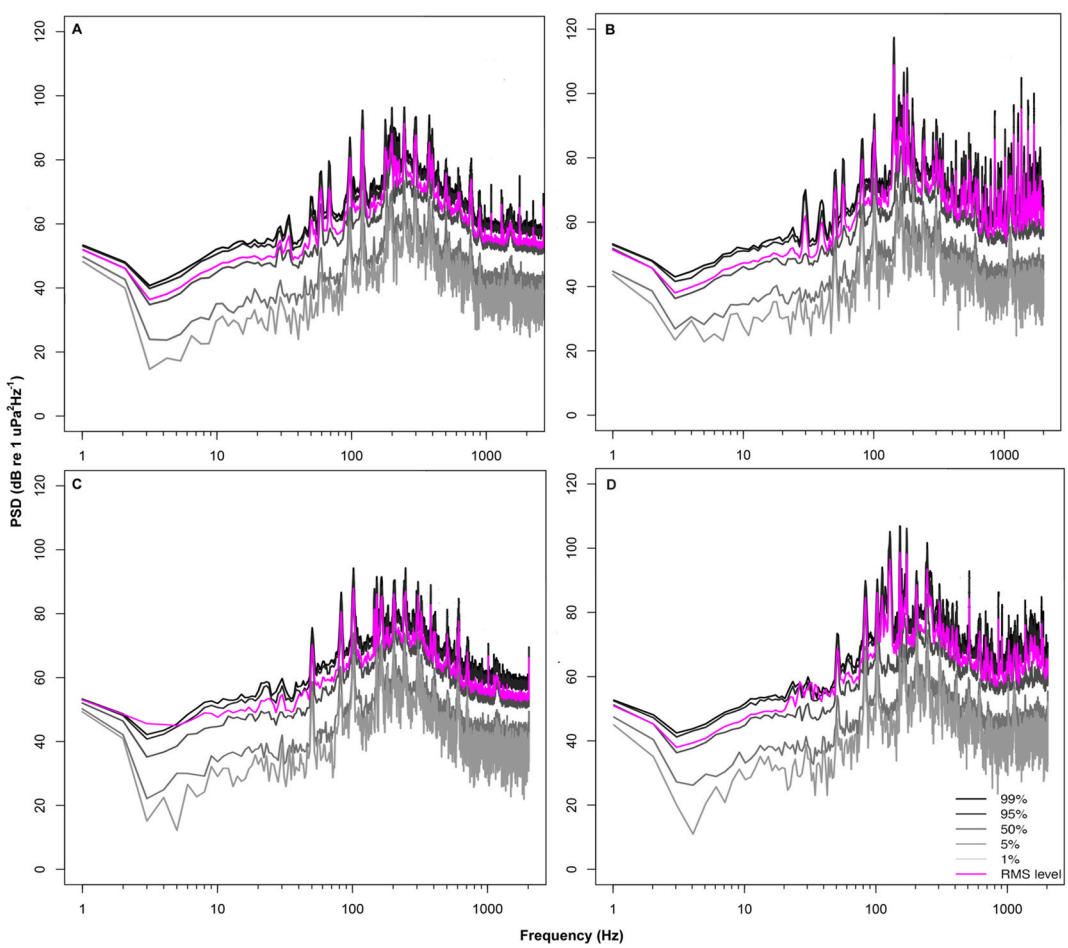

Fig. 2. Diagram of treatments' noise condition. Power Spectral Density (PSD) for each treatment, A = control, B = added noise, C = predator presence, D = added noise + predator presence. Recorded sounds (C = 8, N = 6, P = 4, NP = 6) were concatenated to form a single contiguous spectrogram. The plots depict the different percentiles (1%, 5%, median, 95%, 99%) and the Root Mean Square level (RMS). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

3. Results

YOY lobsters (total = 134) were randomly assigned to four conditions in a daytime or nighttime experiment: control (C = no predator nor noise), added noise (N), predator presence (P), and added noise + predator presence (NP). Recorded sounds (C = 8, N = 6, P = 4, NP = 6) were concatenated to form a single contiguous spectrogram for each treatment. The noise levels were measured with control and treatment tanks running concurrently, in order to ensure there was no cross-contamination of noise between tanks. The added noise treatment consisted of a low-frequency multi-tone sound with sound pressure level approximately 20 dB above non added noise (control and predator) conditions between frequencies 100–200 Hz (Fig. 2).

3.1. Lobster behavior

The primary focus of the study was the different behavioral patterns (see methods section) and the relative percent time engaging in a specific behavior. As expected for YOY lobster life stage, the most common behavior was hiding (daytime: 81.93 [15.41]% of total time, median [IQR]; nighttime: 80.50 [22.13]% of total time) in the substrates (Fig. 3; *i.e.*, lobsters were not visible on video recording). Lobsters' hiding behavior decreased significantly by 13% during the nighttime under the combination of added noise and predator presence than under any other tested conditions. During daytime there was no evidence of any treatment affecting lobster hiding behavior.

When lobsters were not hiding (nighttime), they were engaged in shelter construction (3.5 [8.6] %), peeking (7.8 [9.2] %) and exploration (3.3 [8.8]%) (Fig. 4). We found no evidence that added noise (N) and predator presence (P) alone influenced lobster behavior during nighttime or daytime. However, under the combination of predator and noise during nighttime, YOY lobsters were significantly more active, exploring approximately 2-fold more than in other control and noise conditions (Fig. 4).

3.2. Substrate choice

Substrate choice was assessed twice, at the beginning (substrate chosen when released into the experimental tank, after 5 min acclimation) and at the end of each trial (substrate where lobsters were found after 3 h). YOY lobsters in absence of noise and predator did not have any particular preference for rock or oyster shells, contrary to those exposed to either noise or predator, or both in combination where there was a preference for rocks (Fig. 5). In control conditions, no initial preference between rocks or oysters was observed, irrespective of time of day. Rocks was the preferred final choice under all conditions in both diurnal and nocturnal trials. All lobsters chose to hide among rocks or oyster shells within the first 5 min, time lobsters spent within each substrate did not significantly differ among treatments (Fig. S3), or the number of times lobsters switched between substrates (Fig. S4). No lobsters were observed settling on the bare sand in the video-recordings and after 3 h.

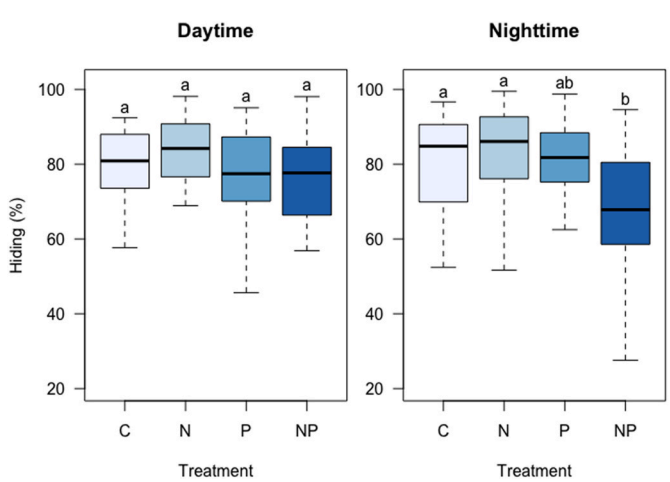

Fig. 3. Diel boxplots of hiding behavior. Comparative results of time (in %) young-of-year lobsters spent hiding during daytime and nighttime under (C) control, (N) added noise, (P) predator presence and (NP) added noise + predator presence treatments. Horizontal bars in the boxplots indicate the median; upper and lower edges of the rectangles show the first and third quartiles, respectively; vertical error bars extend to the lowest and highest values in a 1.5-fold inter-quartile range (R Core Team, 2021). Different letters (a,b) indicate significant differences. A notation with more than one letter (a,b) means that there is no significant difference with either (a) or (b) (Tukey's post-hoc test, $p < 0.03$).

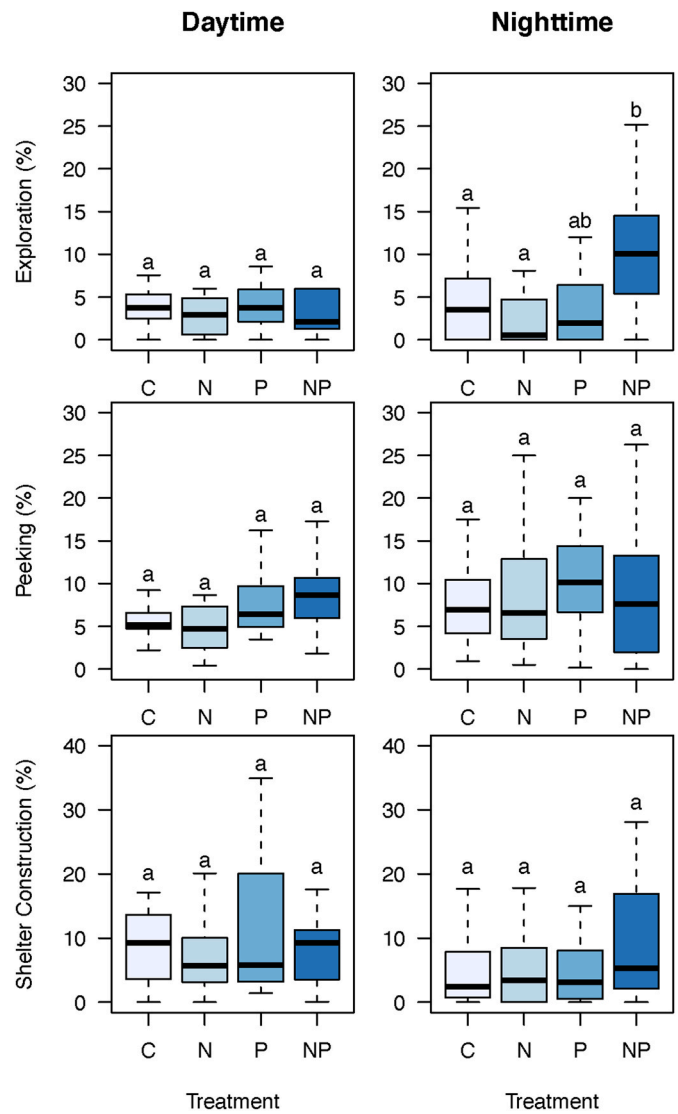

Fig. 4. Diel boxplots of active behaviors. Comparative results of time (in %) young-of-year lobsters spent in active behaviors (exploration, peeking, shelter construction) during daytime and nighttime under (C) control, (N) added noise, (P) predator presence and (NP) added noise + predator presence treatments. Horizontal bars in the boxplots indicate the median; upper and lower edges of the rectangles show the first and third quartiles, respectively; vertical error bars extend to the lowest and highest values in a 1.5-fold inter-quartile range (R Core Team, 2021). Different letters (a,b) indicate significant differences. A notation with more than one letter (a,b) means that there is no significant difference with either (a) or (b) (Tukey's post-hoc test, $p < 0.03$).

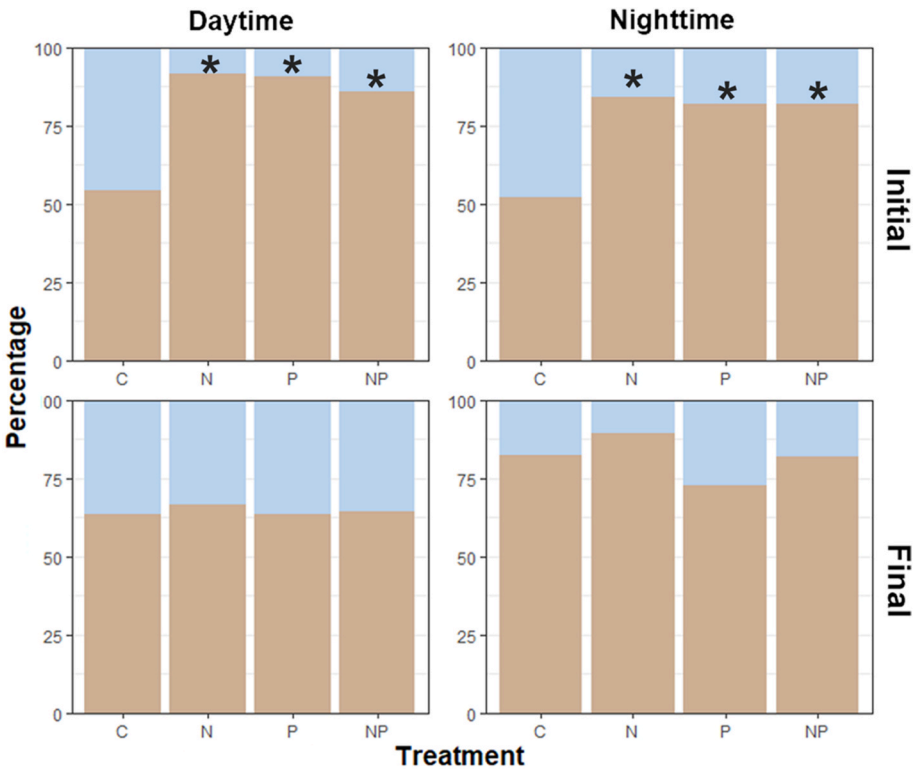

Fig. 5. Substrate choice of young-of-year lobsters. Initial and final choice were assessed after 5 min acclimation and 3 h of exposure, respectively. Treatments are (C) control, (N) added noise, (P) predator presence and (NP) added noise + predator presence. Colors represent the substrates, brown = rocks and blue = oyster shells. Stacked bars with asterisks are significantly different from the control condition (Holm-Bonferonni correction see Table S4). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

We have demonstrated how the joint effect of added low-frequency noise and predator presence modified YOY lobsters' behavior. With potential ecological consequences, YOY lobsters under the added noise and predator presence treatment spent more time outside their substrate (shelter), exploring, and less time hiding. Increase in time spent outside may be correlated with higher mortality in small juvenile lobsters as shown in previous studies with juvenile American lobsters and predatory green crabs (*C. maenas*) (Rossong et al., 2011). An explanation for this exploration under noise and predator presence treatment could be described by the "distracted prey hypothesis" (Chan et al., 2010). This hypothesis implies anthropogenic noise may reallocate the organism's finite attention, distracting them, and therefore preventing them to respond to predatory threats. A similar reaction was observed in other taxa in both aquatic (Voellmy et al., 2014) and terrestrial habitats (Dukas, 2004). For example, an experiment done on European seabass (*Dicentrarchus labrax*) tested the fish's response to a rapidly increasing piling noise (Spiga et al., 2017). The seabass reacted with startle responses to piling noise, which led to an increased turning behavior and less time spent in the safe zone (zone of the tank furthest from the predator). While the fish appeared to exhibit increased vigilance, they also reduced their predator inspection behavior. Studies focused on the behavioral impact effect of anthropogenic noise on decapods are limited. However, research on *C. maenas* has led to the distinction of stage-specific responses to noise (Carter et al., 2020, Wale et al., 2013b). Ship noise exposure in juvenile crabs increased the time for them to seek shelter after a simulated attack, like the adult crabs (Wale et al., 2013a), but it made juveniles less likely to respond to predator threats (Carter et al., 2020). To our knowledge, the current study is the first focused on European lobster juvenile behavioral response to the joint effect of predator presence and added noise. Interestingly our findings are similar to *C. maenas* juvenile's lack of response to predator threats, YOY lobsters demonstrated a decreased antipredator behavior (i.e., hiding) and increased exploration behavior. The increase in exploration behavior seen in YOY lobsters under the joint effect of noise and predator could be attributed as an indicator of stress, as lobster might have attempted to avoid the noise and perceived it as a threat (Aimon et al., 2021; Stoner, 2012). Aimon et al. (2021) recent experiment revealed that *C. maenas* increased its activity and antennae beats when exposed to seabed vibrations of 20 Hz frequency, indicating that anthropogenic underwater vibration could induce a behavioral response and cause higher stress levels. Nevertheless, YOY lobster under noise and predator presence treatments alone did not increase their exploration behavior. Our interpretation is that the stressors separately did not have the magnitude to elucidate a significant behavioral response, while joint exposure (noise and predator presence) led to an additive stress level effect expressed in increased activity.

An alternative explanation for the lobsters' higher exploration activity under the noise and predator presence treatment, could be that noise masked the auditory cues that lobsters perceived from the predator. Our added noise may be modest in comparison with other anthropogenic noise sources, but it produces a tonal sound that overlaps with lobster's natural environmental signals for predator detection and 'buzzing' sounds (low-frequencies ~100 Hz) produced by adult *H. gammarus* (Jézéquel et al., 2018). As animals rely on multiple senses, it is difficult to fully understand the multimodal impact of sensory pollutants on animal behavior and perception. It is known that lobsters principally use olfactory and visual cues to detect predators (Wahle, 1992) and food (Mackie, 1973), but as noise exposure has been demonstrated to reduce foraging behavior in decapods (Wale et al., 2013a), the higher exploration activity might not be related to masked auditory cues or an urgent food craving.

Our results also demonstrated that the single and joint effect of noise and predator presence influenced initial substrate choice (rocks or oyster shells) of young-of-year (YOY) *H. gammarus* lobster. Overall, rocks were the preferred substrate, but oyster shells also appeared to be a favorable substrate providing habitat where YOY lobsters can hide. In the control conditions, lobsters did not have a significant preference for rocks over oyster shells in their initial choice (first substrate chosen after 5 min acclimation). It was exclusively after exposure to predator presence and/or added noise that the majority of lobster preferred to hide in

the rocks as their initial choice. To date, there are no studies linking substrate choice with stress responses in European lobsters. Nevertheless, an experiment on captive juvenile European spiny lobsters (*P. elephas)* explored how the physical properties (e.g. shape, size) of shelters influenced the animals' choice (Gristina et al., 2009). Contrary to our results, *P. elephas* preferred shelters with holes closer to body size under control conditions and had no shelter preference in predator presence. In our results, YOY lobsters' initial substrate selectivity under added stressors might be a response to a potential threat. Preference for rocks may be based on their geometry (size, shape and weight) which provided wider and more stable spaces where lobsters could change direction easier within the substrate. The substrate providing shelters offered in our experiment did not simulate the complexity of a rocky benthic community or a European oyster reef. However, they complied with their refuge function similar to natural hard substrate and interstitial spaces where juvenile lobster sought shelter. If repopulation of *O. edulis's* reefs in the German Bight were to be successful, these habitats are worth assessing since every possible addition of hard substrates in the area could potentially lead to an increase in lobster's survival.

Marine noise pollution research frequently focuses on the effect of short-lived intense noise (seismic surveys, offshore wind farm construction, pile drilling) on marine taxa. Here, we were interested in demonstrating that a subtle constant noise also poses a significant risk. It is ecologically important to set a baseline to sound levels that can elucidate a response in animals, this can help in noise pollution mitigation efforts as well as improving aquaculture methods. The added noise in our experiments is comparable to ocean traffic noise, as reported in generalized "ambient" underwater noise spectra in the sea (Wenz, 1962). The results from in-tank laboratory experiments are always hard to extrapolate to the field, and this is especially true for experiments using sound. One of the many constraints might be the presence of pressure release boundaries that would transfer part of the sound energy across the boundary (mainly at water surface) and reflect the other part into the original medium (effect accentuated here by the tank walls). However, one strength of our study is the use of a cheap and easy to replicate sound source that can increase the amount of data currently available on the effects of noise pollution (de Jong et al., 2017). Our lobsters were not reared in pristine soundscape conditions (see Fig. S5), as they were subject to aquaculture settings (Slater et al., 2020). However, they were naive to predator presence, which combined with noise, uncovered the negative impact noise has on them. We could not include historical noise and direct predator exposure, as lobsters were borrowed and were aimed for conservation purposes. We note further work is needed to understand European juvenile lobster's sensitivity to substrate vibration and particle motion. There are several studies (see Table S5) and key review articles specialized in particle motion components (Nedelec et al., 2016; Roberts and Elliott, 2017; Popper and Hawkins, 2018; Hawkins et al., 2021) which can provide guidance for future studies tackling these components.

5. Conclusion

This paper is the first to test the joint and separate effect of added noise and predator presence on substrate preference and antipredatory responses of YOY European lobsters. As current understanding of how underwater noise may synergize with other stressors is limited, our research is fundamental. While the results show that noise influences the antipredator behavior of YOY *H. gammarus*, it is not yet clear if this will indeed have an adverse effect on the lobster's survival. Care is needed when interpreting our predator-prey interaction, as our predator was restrained. Furthermore, the effects of long-term noise exposure and the potential of sensitization, habituation and/or possible long-term damage that could persist even after exposure ended are still unknown. However, if low-frequency noise disrupts antipredatory behavior in YOY lobsters increasing their vulnerability, its effects could resonate in the overall lobster stocks. This outcome pinpoints the importance of including key benthic community relationships in anthropogenic noise risk assessments. Among others, effects of noise must be taken into consideration in plans involving the multi-use of any offshore area for decapods' stock enhancement, aquaculture, and temporary no-take zones.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118108.

Author contributions

Laura Leiva: Conceptualization, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Sören Scholz**: Investigation, Data curation, Writing – review & editing. **Luis Giménez**: Conceptualization, Data curation, Writing – review & editing. **Maarten Boersma**: Conceptualization, Resources, Writing – review & editing. **Gabriela Torres**: Conceptualization, Investigation, Writing – review & editing. **Roland Krone**: Conceptualization, Resources, Writing – review & editing. **Nelly Tremblay**: Conceptualization, Investigation, Data curation, Writing – original draft, Writing – review & editing.

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CHAPTER 5. GENERAL DISCUSSION

The main aim of my thesis was to investigate the impact of anthropogenic stressors on the early life stages of European lobsters on the island of Helgoland to understand the status of the lobster population, their future and draw awareness towards lobster conservation. An approach of field sampling and laboratory experiments was done to evaluate lobster larvae numbers in the wild and the physiological and behavioral effects of anthropogenic stressors (*e.g.* temperature rise, ocean acidification and underwater noise) on the early life stages of European lobsters. Below, I highlight the main results from this thesis and discuss the possible consequences of ocean warming and acidification for lobster larvae according to their physiological responses. Additionally, I discuss the impact of a low-frequency noise on juvenile lobster's behavior, which highlights the importance of including benthic invertebrates in any noise risk assessments.

5.1 Helgoland's lobster larvae population

The small size of the population of lobster larvae and adult lobsters around the island of Helgoland has been a concern to the Helgoländers (Helgoland inhabitants) and scientist inhabiting the island for the last past decades. Therefore, money, resources and time has been invested in lobster restocking, leading to the release of 14,000 juvenile lobsters between 2000 and 2017 by Alfred-Wegener-Institut (AWI) Helmholtz-Zentrum für Polar-und Meeresforschung-Biologische Anstalt Helgoland and Reefauna. Furthermore, additional fishing regulations such as an agreement on minimum landing size (85 mm carapace length) and a ban on fishing berried females were implemented. However, based on fishermen logbooks the annual catches remain low at 200-300 lobsters caught annually (Schmalenbach et al., 2009; Schmalenbach and Franke, 2010). Estimation of population sizes were calculated in 2007 and 2008 and varied between 21,000 and 29,000 lobster respectively (CL > 58 mm) (Schmalenbach, 2009). Berried females are frequently seen in yearly lobster monitoring done by AWI, but information on successful recruitment of lobster larvae is to date unavailable.

Principally, because of the challenges of sampling early life stages of European lobsters around the island of Helgoland.

In chapter two, I evaluated the use of light traps for sampling lobster larvae in the German Bight. AWI's Helgoland Roads long time-series plankton sampling have seldomly caught lobster larvae, even though it is a high frequency sampling (every Monday, Wednesday and Friday) running since 1974 (Greve et al., 2004). Contrastingly, studies in the other areas with *Homarus* lobsters have reported catching lobster larvae using plankton nets (Scarratt, 1973; Fogarty, 1983). Therefore, the aim of the study was to explore another method to catch lobster larvae around Helgoland. Tube light traps have been effective in catching crustacean larvae, and specifically lobster species of the *Homarus* genus (Øresland, 2007; Sigurdsson et al., 2014). The light traps used in my experiment were slightly modified based on suggestions from Sigurdsson et al. (2014), to optimize handling of the traps and retention of larvae. The traps were tested with stage I larvae and were successful in catching lobster larvae in laboratory experiments at different volumes (100 and 5000 liters). However, when tested in the field the light traps did not catch lobster larvae. The major reasons I believe the sampling was unsuccessful is the numbers of larvae are low and limited to certain areas. Based on the work of Schmalenbach, Mehrtens, Janke, & Buchholz (2011), the restocking of juvenile lobster can have a positive effect in increasing the lobster population, if at least 10,000 juveniles are released every year (Schmalenbach et al., 2011). Presently, 2000-4000 juvenile lobsters are released every year by the lobster conservation company, Reefauna.

Choosing the correct deployment sites for the traps is an additional challenge. The sampling sites in my field study were chosen because they are suitable lobster habitats made of rocky seafloor and recommended by local sailors and fishermen. However, possibly the following locations: North (Nordmole) and South (Nebelhorn) of the island may not serve as nursing grounds for European lobster. Other reason could be the strong tides and currents the island is subject to make it difficult to catch lobster larvae. Moreover, the unique behavior observed by Schmalenbach and Buchholz (2010) may also play a role in the difficulties of finding lobster larvae in the field. This behavior is the abrupt and early change in larval phototactic response to light which may prevent larvae from drifting away from the suitable environment (Schmalenbach and Buchholz, 2010). Considering these sudden changes in

phototactic response traps were deployed at different depths, close to the seafloor and at the top of the water column. Additionally, placing the traps at different depths can help gather further information on the vertical distribution of lobster larvae in the water column.

My field experiment was conducted for one summer season, and as the Helgoland roads have shown catching a lobster larva may take years without an adequate trap. Since lobster larvae are attracted by light during stage I principally, I believe further sampling around the island using light traps is necessary. This should be implemented like the adult lobster monitoring done twice a year by AWI around Helgoland. The lobster monitoring is done during a week span, lobster pots are deployed and retrieved the next day during three consecutive days. Lobster larvae sampling could also be performed twice a year during larvae hatching season (May to August) (Mehrtens, 2011). To reduce time and labor, I suggest leaving the traps for longer periods, possibly three days and repeating the sampling three times in May and in August. It would also be interested to examine different habitats around the island, ranging from sandy to rocky seafloor to have a better idea of lobster larvae preferred habitats. Unfortunately, there is no dedicated fixed program yet, even though several PhD projects have focused on lobster biology and contributed to elucidating more information on a key organism of the North Sea benthic community (Schmalenbach, 2009; Mehrtens, 2011). However, these findings come every 5 to 10 years, leaving gap years when lobsters are not studied. The lack of continuity makes it hard to continue gathering information on the fitness of Helgoland's lobster and the population numbers. Currently being up to date and knowing how lobster are coping with climate change is time sensitive and would be more efficient if there were constant monitoring and research. I believe it would be necessary to have a fixed working group focused on lobster ecology, that can be aided throughout the years by students, PhD candidates and postdocs of AWI in collaboration with the established rearing lobster facilities of Reefauna.

5.2 Anthropogenic stressors in the North Sea

The main overreaching question of my thesis was to investigate how anthropogenic stressors are impacting Helgoland's lobsters. I chose the most threatening stressors based on the geographical location, biology of clawed lobsters and literature review. A study focused on the North Sea – Baltic Sea transition zone revealed the top stressors of the area are: nutrients, climate anomalies, non-indigenous species, noise and contaminants (Andersen et al., 2020). The effect of nutrient-rich freshwater pulses in the North Sea is a factor that has a great effect on phytoplankton communities which are closely dependent on macro-nutrients availability for growth. However, the development and survival of larvae and juvenile lobsters are principally influenced by temperature and other abiotic factors like salinity and seawater pH (Charmantier et al., 1991b; Charmantier and Mounet-Guillaume, 1992; Anger et al., 2020). Therefore, climate change is one of the stressors with potentially higher impact on European lobsters. Especially, coastal marine ecosystems of the North Sea that have been shown to experience greater ocean warming than other habitats (Wiltshire et al., 2010). Hence, in chapter tree I focused on larval development under a temperature gradient and ocean acidification.

The impact of anthropogenic activities on marine ecosystems can be determined based on the intensity, duration and the pressure the activity is causing on a specific ecosystem (Andersen et al., 2020). Industrial activities for example consist of long-term projects that involve highly disruptive construction sites and long-term operations. As the North Sea is a commercially developed area, I chose to focus on noise, one of the by-products of industrialization (Newhall et al., 2016; Erbe and McPherson, 2017; Duarte et al., 2021). Offshore windfarms (OWF) for example are a source of constant noise due to turbine operations and understanding the impact this noise can have on species that may find a home in OWF foundations is pertinent (Tougaard et al., 2008, 2020; Pine et al., 2012). Therefore, in chapter four, I used a multi-stressor approach to test the joint and separate effect of added noise and predator presence on substrate preference and antipredatory responses of juvenile European lobsters.

5.2.1 Rising temperature and decreasing pH: lobster larvae and future climate change scenarios

As meroplankton, decapods make up a great portion of both benthic communities and planktonic fauna in the water column. Furthermore, they represent a mayor food source across the marine food web, as well as a vital source of high-quality protein for people (Tidwell and Allan, 2001). However, the Anthropogenic Ocean has been subject to human activities which have caused a change in marine abiotic parameters (IPCC, 2021), and crustaceans are directly affected by increasing pCO_2 levels and rising temperatures (Whiteley, 2011; Lopez-Anido et al., 2021; Niemisto et al., 2021). Assessing the impact of environmental change on animals with complex life-stages is challenging due to the variety of responses from one developmental stage to the other. Moreover, for animals with long-life spans, like clawed lobsters; following somatic growth under ocean acidification would take decades of monitoring. Therefore, most studies focus on selected life stages (*i.e* larval, juvenile or adults) to have a snapshot of how crustaceans will cope with predicted future climate change conditions. It is clear the worst-scenarios predicted by the IPCC may pose a threat for the fitness and survival of an essential group of the marine food web and benthic ecosystems (Rato et al., 2017; Niemisto et al., 2021). However, if humans deaccelerate their industrial activities and reduce fossil fuel consumption the negative impact could be diminished.

Although efforts have been made to evaluate the effects of OA and OW (ocean warming) on lobster larvae separately (Arnold, Findlay, Spicer, Daniels, & Boothroyd, 2009; Keppel, Scrosati, & Courtenay, 2012; Noisette et al., 2021; Rato et al., 2017; Schmalenbach & Franke, 2010), few studies have assessed their joint impact (Small et al., 2015; Waller et al., 2017; Niemisto et al., 2021). In chapter two conducted an experiment to examine the joint effect of ocean warming and acidification on survival, development, morphology, energy metabolism and enzymatic antioxidant activity of the larval stages of the European lobster. To provide a more complete picture on the thermal limits of lobster larvae under acidification the experimental setup included a gradient of 10 seawater temperatures (13 – 24°C). I hypothesized there would be a synergistic or interactive effect of elevated pCO_2 (~ 1160 µatm) with elevated temperatures, as several climate change studies on marine invertebrates have suggested (Small et al., 2015; Waller et al., 2017; Villalobos et al., 2020). Additionally, I

expected larvae in the lower temperature treatments, regardless of pCO_2 conditions to have a reduction in instantaneous growth. Interestingly, our results showed no evidence of synergistic effects of temperature and pCO_2 on any of the measured variables. However, the stressors did influence the measured parameters separately. Larvae under the elevated pCO_2 treatment had lower survival rates and significantly smaller rostrum length. While temperature was the main driver of energy demands with increased oxygen consumption rates and elemental C:N ratio towards warmer temperatures, with a reducing effect on development time.

The growth of crustacean larvae is at its best at the temperature at which the physiological performance is optimal. Similarly, growth will decrease at higher and lower optimal temperatures (Anger et al., 2020). The results of my experiment showed lobster larvae are quite resilient to temperature they normally do not experience in their environment. To date seawater temperatures at Helgoland do not surpass 21°C. Nevertheless, the combined results of chapter two showed larvae at the warmest temperatures >21°C appear to thrive (i.e. higher survival and greater biomass). Yet only in two of the parameters measured, did the fitted curves for data points plateau at the warmer end, and start to slightly decrease at 23 and 24°C (Figure 9). Survival rate and rostrum length were the only ones negatively affected by elevated pCO_2. This could suggest the *pejus* point is approximating, and that these two parameters were more sensitive to elevated CO_2 than others measured. Yet to further draw conclusions and pinpoint the mechanisms that caused these changes, further studies are necessary. From a physiological point (*e.g.* higher antioxidant responses and compromised respiration), it would have been interesting to include warmer temperatures to reach the lobster larvae thermal limit.

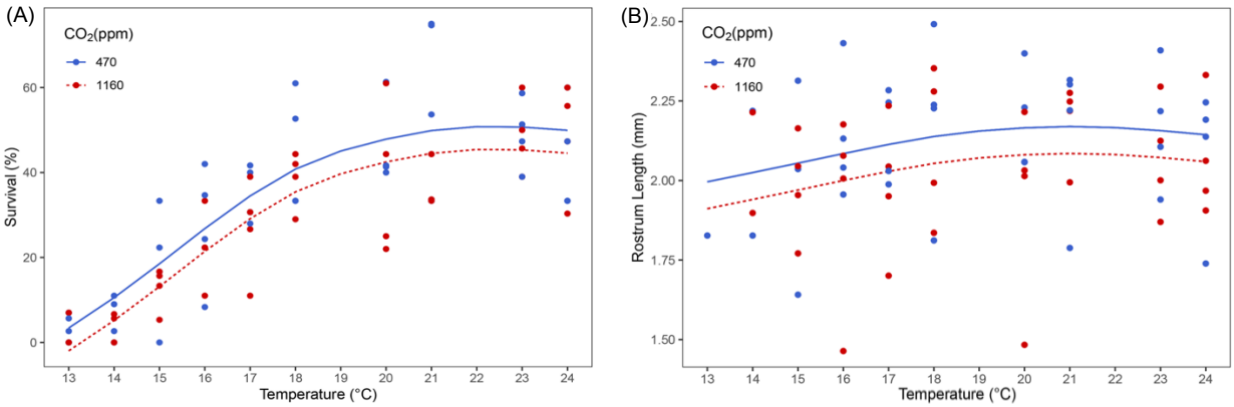

Figure 9. Effect of temperature and ocean acidification on survival and morphology of stage III *Homarus gammarus* larvae. **(A)** Survival and **(B)** rostrum length was influenced by CO_2 and temperature.

European and American lobster larvae can tolerate certain levels of ocean acidification and warming (Schmalenbach and Franke, 2010; Noisette et al., 2021). Species considered "tolerant" to high pCO_2 tend to maintain energy metabolism, metabolic rate and aerobic capacity (Calosi et al., 2013). The up-regulation of metabolic rate arises from the increased energy demand to maintain acid-base homeostasis and other physiological functions (Wood et al., 2008, 2010; Beniash et al., 2010). These changes to keep up with energetic demands may still have an underlying effect on other physiological aspects like thermal sensitivity (Metzger et al., 2007; Walther et al., 2009) and osmo-/iono-regulation (Dissanayake et al., 2010). Thus, I anticipated at warmer temperatures >21°C exposure to elevated pCO_2 would enhance the larvae's sensitivity to heat and thus narrow their thermal tolerance window. Indeed, previous studies on crustaceans and thermal stress revealed elevated pCO_2 can narrow the thermal tolerance of the edible crab, *Carcinus pagurus* and the spider crab, *Hyas araneus* (Metzger et al., 2007; Walther et al., 2009; Whiteley, 2011). Interestingly, none of the physiological end points measured suggest exposure to elevated pCO_2 narrowed the thermal tolerance of lobster larvae. Lobster larvae routine metabolic rate (RMR) increases linearly with increasing temperature independent of pCO_2 treatment and there was no evidence OA had a negative impact on antioxidant enzyme activity (GST, GPx, SOD and CAT).

In my thesis I provide new information on how lobster larvae will cope with the predicted SSP5-8.5 scenario for 2100 in which SST will increase by 2-3°C and atmospheric CO_2

will have reached 1000 µatm. My results suggest that even though at the cellular level (*i.e.* enzyme activity) or physiological level (i.e development time to reach stage III) high pCO_2 did not elicit a response; at the population level (survival) there were significant negative effects. If this low mitigation scenario were to be reached, I believe the future of the Helgoland's lobsters would be as risk of collapsing. However, the SSP5-8.5 scenario was intended to explore a high-risk future in which there are few or low mitigation policies and continued fossil-fuel intensive usage (Calosi et al., 2013). Several studies and climatologist argue the SSP5-8.5 scenario should be labelled the unlikely worst case instead of the commonly used term "business as usual". Suggesting that the modest mitigation and weak mitigation scenarios are more likely to be reached in 2100 (Ritchie and Dowlatabadi, 2017; Ho et al., 2019; Hausfather and Peters, 2020). I also believe mitigations efforts are already in place and will be further developed in the following years up to the end of the century to avoid reaching the worst-case scenario. Nonetheless, studies showing the consequences of the SSP5-8.5 scenario, like my experiment are vital to create awareness on the effect climate change may have on endangered species.

5.2.2. Effects of underwater noise pollution on young-of-year lobsters

Despite the increase in anthropogenic noise due to the exploration of natural resources in the ocean, its effect across different taxa and life stages is far from being described (Metzger, Sartoris, Langenbuch, & Pörtner, 2007; Walther, Sartoris, Bock, & Pörtner, 2009; Whiteley, 2011). Invertebrates, including crustaceans have received limited attention, even though their abundance is critical for ecosystem functioning (Hawkins et al., 2015). Moreover, the interactions between noise and other stressors and its impact on crustaceans' behavior is relatively unknown (Tidau and Briffa, 2016). In chapter four, I address this knowledge gap by using a factorial behavioral analysis to evaluate whether synergies exist between noise and predation. Additionally, I examined whether noise is an additional stressor that alone or in combination with predator presence has an influence on substrate choice and behaviors associated with survival. The intend of the experimental set-up was to simulate a North Sea benthic ecosystem, providing young-of-year lobsters their preferred habitat: rocks and reef-type habitats made of small crevices (i.e. oyster shells) (Able et al., 1988; Lawton and Lavalli,

1995). The green crab *Carcinus maenas* was chosen since it's a recognized predator of YOY lobsters and commonly found in the subtidal habitats of the German Bight (Mercer et al., 2001; Rayner and McGaw, 2019). The assessment of prey-predator relationships and substrate choice relationships in a constant low-frequency noise context can provide insights into natural and artificial hard-substrate community dynamic exposed to low-frequency anthropogenic noise.

My results suggest joint exposure to a low-frequency noise (100-200 Hz) and predator presence increased the amount of time lobsters spend outside their shelter exploring during nighttime. The latter is ecologically detrimental as animals may expose themselves more to predators in natural conditions (Rossong et al., 2011). An explanation for YOY lobsters increased exploration outside their shelters in the presence of a predator may be described by the "distracted prey hypothesis" (Chan et al., 2010). This implies noise may reallocate the organism's attention, distracting and thus preventing them to respond to predatory threats. An increase exploration in the noise and predator presence treatment could further be explained by cross-sensory interference. This would entail the interference of an auditory cue, in the experiment it could be the added noise may intervene with an olfactory cue (use to detect predators) (Halfwerk and Slabbekoorn, 2015). However, more research and evidence would be needed to support the cross-sensory interference hypothesis.

The justification of this study was to find if noise affects juvenile lobster behavior, given the efforts to increase lobster stocks around Helgoland and area now populated with OWF. These behavioral responses raise concerns about the effects offshore wind farms operational noise may have on European lobster living among its foundations naturally, or in the frame of restocking, multi-use and temporary no-take zones programs. The impact a low-frequency noise had on YOY lobster may seem minor, since the experimental was limited to behavioral studies. However, I was interested in demonstrating that a subtle constant noise (comparable to ocean traffic and OWF operational noise) also poses a significant risk and can elucidate a response. Additionally, my thesis results can serve as a baseline to sound levels that influence the behavior of YOY lobster, a life-stage that is understudied due to their cryptic lifestyle (*i.e.* shelter-bound). Subsequently, it remains important to continue studying the effects of noise pollution on crustaceans to develop a well-rounded view on the overall impact on marine ecosystems.

5.3 Conclusion and perspective

My thesis focused on an animal that was overfished in the past and is in a vulnerable period, as the population numbers are extremely low in comparison to the early 1900s. The three stressors (ocean acidification, ocean warming and noise), I chose to investigate are relevant both at a local and global level. Locally the major offshore activities around Helgoland are, OWF and constant boat traffic and globally climate change is impacting seawater temperature and pH. I started from the bottom of the lobster population, working with larvae and then with juveniles. Both life-stages are population bottlenecks and mark a key period in the metamorphosis of lobsters from planktonic larvae to benthic juveniles. I integrated my experiments by first focusing on field studies and searching for lobster larvae in the field. Afterwards, I expanded my research and investigated three key anthropogenic stressors experienced by Helgoland's lobsters.

There is a gap of knowledge in the lobster population dynamics of European lobsters on Helgoland. Finding the best way to capture lobster larvae is one of the first step towards knowing more on lobster larvae settling rates and successful recruitment. The light trap developed in my thesis could serve as a tool to catch lobster larvae in the field. However, further studies are necessary to find the correct places to deploy the traps and an intensive yearly sampling should be implemented. Furthermore, climate change is an additional obstacle the European lobsters on Helgoland are facing in the route to recovery. The restocking efforts could be potential stunted by the exponential increase in CO_2 emissions predicted by the low mitigation scenario SSP5-8.5 from the IPCC. My results suggest near-future pCO_2 conditions have a negative effect on lobster larvae survival to stage III. Which underlines the importance of climate change mitigation efforts. As a small island, Helgoland is active in tackling climate changing by using electric cars, recycling and minimizing waste. There is also a future interest in making Helgoland a green hydrogen production site. Yet all these expansions and industrial activities must be done with precaution. For example, studying the effects of noise produced by OWF and boat traffic on marine organisms is crucial to conserve the local fauna.

This thesis provides a comprehensive picture of the effects of anthropogenic stressors on the early life-stages of lobsters. I provided new information on the physiology of lobster larvae under ocean acidification and warming and on the behavior of juvenile lobster under noise and the physiology of lobster larvae under ocean acidification and warming. The key findings of the impact of anthropogenic stressors (i.e elevated pCO_2, temperature, noise) and environmental stressors (predator presence) studied in my thesis ranged from the population level to the cellular level (Figure 10).

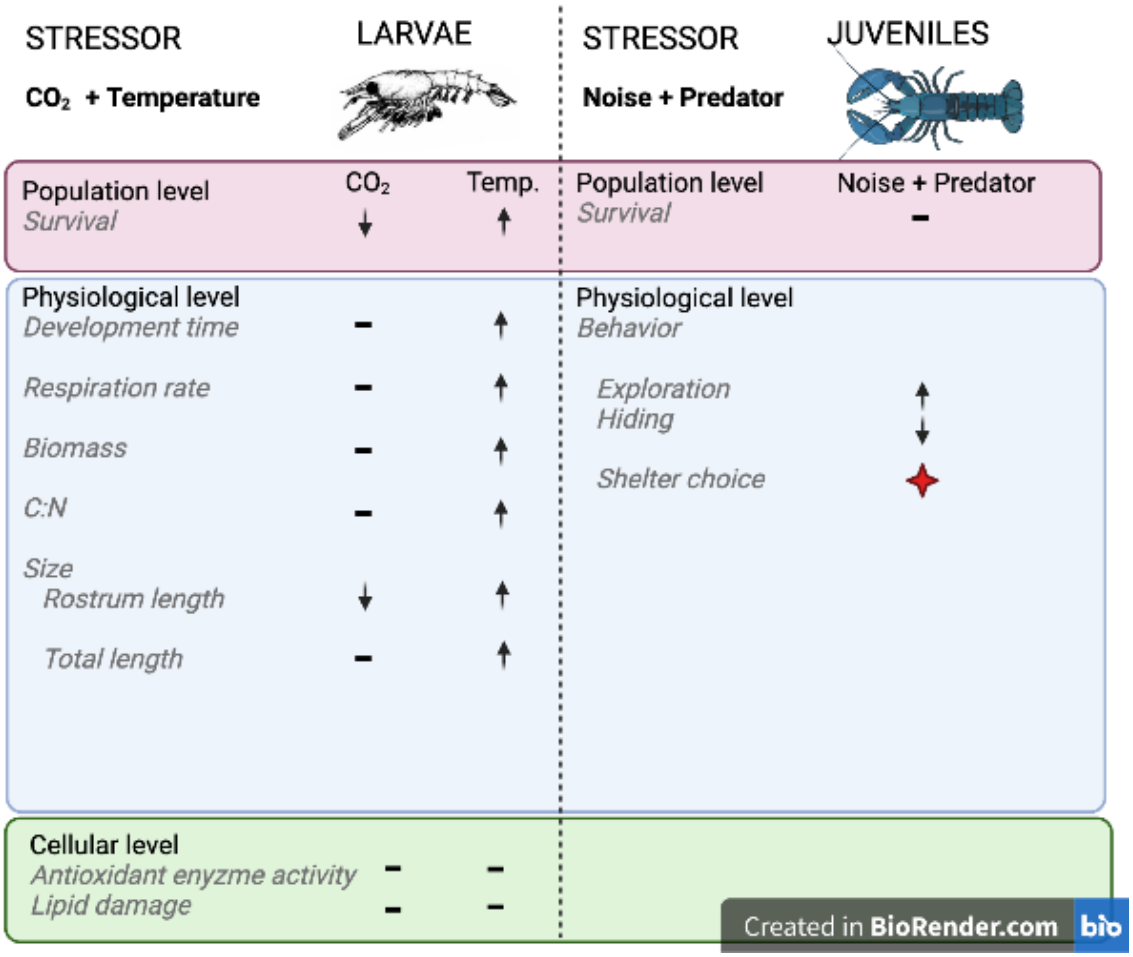

Figure 10. Summary of the impact of anthropogenic and environmental stressors on early life stages of the European lobster. Arrows indicate the direction of the response to increasing CO_2 and temperature and noise + predator presence; (-) means no change detected, and (*) means there was an impact. Created with BioRender.com and stage I – IV larvae modified from nationallobsterhatchery.co.uk.

My results and experiments may provide a first step in investigating the effects of multiple stressors on Helgoland's lobsters. The value of Helgoland's lobster goes beyond economical and ecological value. It also has a cultural value as it is closely linked to the

tradition of lobsterfishers. I believe this could be incorporated more in European lobster research by involving the islanders in citizen science projects. Lastly, special attention should be taken when using vulnerable species to perform experiments. When possible, I would encourage performing experiments that are not as invasive and leave the animals unharmed. As I did on the behavioral study on the effects on underwater noise on YOY lobsters.

5.3.1 Future research

Offshore human activities are on the rise due to the exploitation of natural resources and exhibit no indicative of slowing down. Especially in the present times, political events like the Ukranian war will continue to drive the use of greener energy sources. Germany, in particularly is aiming to depend on greener energy alternatives like offshore wind farms and less on gas an oil. Thus, offshore windfarm (OWF) structures should be utilized in the best ecological way possible. There have been past studies referred to as: “Win-win or win-wind” situations, in which the foundation of OWF are purposed to act as a physical space that offers a new habitat where benthic organisms in particularly could thrive (Kiesecker et al., 2011; Lacroix and Pioch, 2011). Secondly, using OWF as a no fishing zone or temporary marine reserve for local fauna. The construction and operation of OWF have discernable consequences such as an increase in underwater noise. Yet few studies have the permission to enter OWF and conduct research directly in their foundation or measure noise levels.

The next steps for noise pollution research effects on lobsters in the North Sea, would be to conduct studies in the field at OWF, and not only behavioral studies but studies measuring physiological stress. In Helgoland, the lobster conservation company, Reefauna is releasing thousands of juvenile lobsters every year. A small portion of these juvenile lobsters could be released in OWF and periodically be checked by AWI scientists for biochemical stress responses. Lobster hemolymph can be sampled for glucose, total proteins, heat shock protein expression and total hemocyte count as stress bioindicators (Filiciotto et al., 2014). An additional research direction would be to utilize the OWF as an opportunity to allow impacted species, like European oysters to recover and recreate a functioning marine ecosystem (Pogoda, 2019). Oyster reefs offer a habitat for other small benthic animals like shelter-bound

juvenile lobsters, and it would be an interesting project to focus on. As well as a win-win situations for two species under an interest of being recovered.

5.3.2 Lobster conservation

Helgoland's lobsters are a vulnerable population that will continue to be affected by offshore human activity and climate change, therefore restocking programs, scientific support and fisheries management should also be a permanent action.

The subsequent steps in lobster conservation would be to increase the number of juvenile lobsters being released with the support of the state, private funds or NGOs and the Reefauna restocking program. The restocking program is going in the right direction yet more juveniles should be release to have an evident increase in lobster population size (Schmalenbach, 2009). AWI support is key for the monitoring of the lobster population, especially the collaboration of the AWI scientific diving department. Divers could monitor more closely the success of released juveniles in the field, this has not been done in over a decade and would be key to understand juveniles shelter-preferences (Schmalenbach et al., 2011). Understanding lobsters' substrate and shelter preferences could enhanced their survivability success in the field by providing an insight on the best locations to release juvenile lobsters.

The island of Helgoland is doing a formidable job in avoiding overfishing by respecting fishing regulations and incorporating artisanal style fisheries (small boats and few lobster pots). The island controls lobster fishers and few people have a lobster fishing permit, this controls and limits the number of lobsters caught. Therefore, the success of lobster conservation is an effort that has been possible due to locals, scientists and private companies. My thesis is part of these efforts, and one of my goals was to create awareness on the anthropogenic stress Helgoland's lobster are under which are human induced climate change and noise pollution. We can all play a part in reducing our carbon footprint and diminishing the impact we have on our oceans and particularly on vulnerable species and ecosystems.

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