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Interacting climate change effects on mussels (*Mytilus edulis* and *M. galloprovincialis*) and oysters (*Crassostrea gigas* and *Ostrea edulis*): experiments for bivalve individual growth models

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Abstract – The physiological response of two species of mussels (*Mytilus edulis* and *M. galloprovincialis*) and two species of oysters (*Crassostrea gigas* and *Ostrea edulis*) to temperature, oxygen levels and food concentration, factors likely to vary as a result of climate change, was determined experimentally. Bivalves of similar size from different origins were exposed to six temperatures (3, 8, 15, 20, 25 and 30 °C) at two food regimes (2 and 10 µg Chl a L^{-1}) for 6 weeks. In a parallel running experiment *M. edulis* from the same batches were exposed to three different temperatures (15, 20 and 25 °C) and three different oxygen levels (30, 50 and 100%) at two food regimes (2 and >8 µg Chl *a* L^{-1}) for 3–4 weeks. Survival during the experiment ranged from 93% to 100% except for the mussels exposed to 30 °C which showed 100% mortality after three to 32 days. Higher food conditions showed higher optimal temperatures for growth of mussels and oysters. In addition, at the high food treatment, reduced O_2 saturation resulted in lower growth of mussels. At the low food treatment there were no differences in growth among the different O_2 levels at the same temperature. At high food concentration treatment, *M. edulis* growth was higher with low temperature and high oxygen level. Condition index was higher at higher food concentrations and decreased with increasing temperature. In addition, condition was lower at low oxygen saturation. Lower clearance rates were observed at high food concentrations. At 100% saturation of oxygen, mussel clearance rate increased with temperature at High food regime, but not at Low food regime. Mussel clearance rates were significantly reduced with low oxygen concentrations together with high temperature. Oxygen consumption significantly increased with temperature. Oxygen saturation was the main factor affecting mussel clearance rate. High temperature and low oxygen concentration combined significantly reduced clearance rate and increased oxygen consumption. These response curves can be used to improve parameterisation of individual shellfish growth models taking into consideration factors in the context of climate change: temperature, food concentration, oxygen concentration and their interactions. The observation that abiotic factors interact in affecting mussels and oysters is an important result to take into account.

Keywords: *Mytilus edulis* / *Mytilus galloprovinciallis* / *Crassostrea gigas* / *Ostrea edulis* / climate change / growth / temperature / oxygen / chlorophyll

1 Introduction

Two species of mussels and two species of oysters are produced in Europe. In 2018, aquaculture production of blue mussel (*Mytilus edulis*) was highest with 130,000 tonnes, followed by Mediterranean mussel (*Mytilus galloprovinciallis*) and Pacific oyster (*Crassostrea gigas*) both around 100,000 tonnes and European flat oyster (*Ostrea edulis*) with 2500 tonnes (www.FAO.org). Currently, marine seafood represents only 17% of the edible meat, and by 2050, an increase of 36–74% of marine protein is needed to fulfil the world nutritional needs (Costello *et al.*, 2020). Bivalves are amongst the most sustainable animal-source food, still

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Table 1. Number of studies on laboratory experiments with climate-driven environmental factors. Adapted from Catalán *et al.*, 2019;[1] Galley *et al.*, 2010;[2] Rayssac *et al.*, 2010;[3] Ventura *et al.*, 2016;[4] Bechmann *et al.*, 2011;[5] Gazeau *et al.*, 2010;[6] Brenko and Calabrese, 1969;[7] LeBlanc *et al.*, 2005;[8] Tremblay *et al.*, 2011;[9] Rajagopal *et al.*, 2005;[10] Thomsen *et al.*, 2013;[11] Waldeck and Larsson, 2013;[12] Honkoop and Beukema, 1997;[13] Loo, 1992;[14] Beaudry *et al.*, 2016;[15] Fokina *et al.*, 2015;[16] Huning *et al.*, 2013;[17] Berge *et al.*, 2006;[18] Fitzer *et al.*, 2015;[19] Hiebenthal *et al.*, 2013;[20] Riisgård *et al.*, 2014, [21] Sanchez Lazo and Pita 2012;[22] Mesas and Tarifeño, 2015;[23] Kurihara *et al.*, 2009;[24] Range *et al.*, 2014;[25] Jansen *et al.*, 2009;[26] Tomanek *et al.*, 2012;[27] Gestoso *et al.*, 2016;[28] Kheder *et al.*, 2010;[29] Rico-Villa *et al.*, 2009;[30] Timmins-Schiffman *et al.*, 2013;[31] Dineshram *et al.*, 2012;[32] Harney *et al.*, 2016;[33] Kurihara *et al.*, 2007;[34] Petton *et al.*, 2013;[35] Ren *et al.*, 2000;[36] Davis and Calabrese, 1969;[37] Davis and Ansell, 1962;[38] Prado *et al.*, 2016;[39] Rodstrom and Jonsson *et al.*, 2000;[40] Haure *et al.*, 1998;[41] Child and Laing, 1998;[42] Buxton *et al.*, 1981.

Species	Life stage	Temperature (T)	Oxygen (O2)	pH	Salinity (S)	TxO2	TxpH	TxS
Mytilus edulis	Larvae	2[1,2]		3[3,4,5]				1[6]
	Juvenile	3[7.8.9]		1[10]				
	Adult	5[11,12,13,14,15]		4[16,17,18,19]	1[20]		2[18,19]	
Mytilus galloprovincialis	Larvae	2[21,22]		1[23]				
	Juvenile			1[24]	1[24]			
	Adult	1[25]			1[26]		1[27]	
Crassostra gigas	Larvae	2[28,29]		4[30,31,32,33]			1[32]	
	Juvenile	1[34]						
	Adult					1[35]		
Ostrea edulis	Larvae	1[36]			1[37]		1[38]	
	Juvenile	3[39,40,41]						1[39]
	Adult					1[42]		

underexploited and considered part of food for future (Parodi *et al.*, 2018) and fulfil many of the UN sustainable development goals (UN General Assembly, 2015). However, increasing production under climate pressure might be a challenge due to the strong interaction of the bivalves with their environment. Mussel and oyster culture is currently mainly dependent on natural recruitment, which in turn, is influenced by environmental factors such as food supply and water temperature and salinity. The bivalve shellfish are not provided food in culture but feed naturally, taking phytoplankton directly from the water column and do not require feeding, thus production is dependent on the environmental conditions. The main physical factor influencing its distribution is temperature (Seed, 1976) which affects the survival and growth of both adults and larvae. Bivalves are sensitive to climate change induced changes in temperature and salinity, which affect behaviour, physiological rates and the immune system (Matozzo *et al.*, 2012).

Recently, existing evidence of impacts of climate change on aquaculture has been summarised by the FAO (Barange *et al.*, 2018). The main conclusion was that short-term climate change impacts on aquaculture can include losses of production and infrastructure arising from extreme events such as floods, increased risks of diseases, parasites and harmful algal blooms. Long-term impacts can include reduced availability of wild seed as well as reduced precipitation leading to increasing competition for freshwater. Climate driven changes in temperature, precipitation, ocean acidification, incidence and extent of hypoxia and sea level rise, amongst others, are expected to have long-term impacts in the aquaculture sector at multiple scales. Climate change-related stressors (and their interaction) such as increased average temperature, acidification and hypoxia can impact growth, and extreme temperatures can impact survival. This in turn can affect productivity.

Catalán *et al.* (2019) compiled available data on climate-driven environmental factors from previously published experiments conducted on selected aquaculture fish and shellfish species in marine and inland waters. To be added to the database, the studies needed to test the effect of temperature, pH, salinity or oxygen, or their interactions in a laboratory experiment with different concentrations or levels. A total of 42 studies on mussels (*M. edulis* and *M. galloprovincialis*) and oysters (*C. gigas* and *O. edulis*) were selected for a gap analysis in Catalán *et al.* (2019). Of these, 20 studies concerned *M. edulis*, 7 studies *M. galloprovincialis*, 8 studies *C. gigas* and 7 studies *O. edulis* (Tab. 1). The majority of the studies concerned single effects of temperature (20) followed by effects of pH (14) and salinity (4) with no laboratory studies on the effect of oxygen concentrations. Interactions were only studied for temperature and oxygen (2), temperature and pH (5), temperature and salinity (2). The most common response studied was growth, followed by mortality and physiology. The analysis revealed the need for continued research quantifying how changes in interacting abiotic factors affect mussels and oysters.

In order to predict climate change impacts on shellfish, existing formulations need to be adapted in the models. This applies to functional responses to changes in climate change related stressors such as temperature, oxygen, and chlorophyll a (generally considered a good proxy for food for bivalve shellfish). The aim of the present study is to provide data that can improve parameterisation of individual shellfish growth models taking into consideration factors in the context of climate change: temperature, food concentration, oxygen concentration and their interactions. The physiological response of two species of mussels (*M. edulis* and *M. galloprovincialis*) and two species of oysters (*C. gigas* and *O. edulis*) to temperature, oxygen levels and food concentration was determined experimentally. The growth

performance, survival and physiology (clearance rate and oxygen consumption rate) were monitored. These results can be used to parameterise functions describing the physiological response of mussels and oysters to varying environmental conditions for individual growth models. Individual growth models feed into farm-scale biological models to project changes in productivity as a result of climate change (Cubillo *et al.*, 2021).

2 Methodology

Two experiments were carried out. Bivalves of similar size from different origins (*M. edulis* from the Netherlands, *M. edulis* from Denmark and *M. galloprovincialis* from Portugal, and *O. edulis* and *C. gigas* from the Netherlands) were exposed to six temperatures (3, 8, 15, 20, 25 and 30 °C) at two food regimes (2 and 10 μg Chl a L^{-1}) for 6 weeks (Temp/Food Experiment). The temperature range used in this experiment aimed at testing the extremes in order to provide response curves for modelling much smaller effects of climate change. In a parallel running experiment the same *M. edulis* from two origins (the Netherlands and Denmark) were exposed to three different temperatures (15, 20 and 25 °C) and three different oxygen saturation (30, 50 and 100%) at two food regimes (2 and >8 μg Chl *a* L^{-1}) for 3–4 weeks (Temp/Food/Ox Experiment). In both experiments, each combination was carried out in triplicate. The growth performance, survival and physiology (clearance rate and oxygen consumption rate) were monitored.

2.1 Experimental designs

The experimental design of the Temp/Food Experiment consisted of 12 basins of 500 L holding three replicate tanks of 30 L each and ten bivalves per species and origin in five separate nets per tank (Fig. 1a). In October 2016, juvenile mussels were obtained from a Seed Mussel Collector in the Oosterschelde estuary (The Netherlands NL) and longline farms in Limfjorden (Denmark DK) and off the coast of Sagres (Portugal PT). Juvenile oysters were purchased from the hatchery of Roem van Yerseke (The Netherlands). Upon arrival, similar sized shellfish were placed in the set-up for acclimation at 15 °C. Initial sizes are presented in Table 2. The shellfish were placed in small nets per species and origin. The five nets with 10 shellfish each were placed together in a 30 L tank to ensure that all shellfish experienced the same food and temperature conditions during the experiment. Each set of three tanks was placed in a 500 L basin filled with filtered seawater. Aeration was provided to each tank. The basins with tanks and shellfish were distributed over two climatized rooms of Wageningen Marine Research. Each basin was covered with a plastic dome to prevent evaporation of the seawater. After an acclimation period of 3 weeks at 15 °C the temperature of the basins was raised or lowered to the desired temperature over a period of 1 week. Four basins were placed in a room set at 15 °C and two basins in a room set at 8 °C. The other temperatures (3, 20, 25 and 30 °C) were realized through heating or cooling of the water in the basins with a TECO (Bain Marie method). Three times a week seawater in the tanks was replaced by new water that had been acclimated to the right temperature in the basin. Food consisted of algae (*Skeletonema costatum* cultured in outdoor raceways and *Isochrysis galbana* cultured in plastic bags, both bought from the hatchery of Roem van Yerseke. Algae were supplied continuously by pumping a 50% mix of the algae cultures from a bucket placed in the basin to the tanks in the basin with a peristaltic pump. The daily amount of algae needed was calculated based on cell counts of the cultures and a calibration of the cell number and chlorophyll concentration. In addition to the number of liters to reach the initial concentration of 2 and 10 μg Chl a L^{-1} in the 30 L tanks, extra liters were added based on a clearance rate of 1l/h for each individual bivalve.

The experimental design of the Temp/Food/Ox Experiment consisted of 54 × 9.5L insulated tanks with 31 mussels in each and covered by a plastic film to limit gas exchanges (Fig. 1b). In October 2016, juvenile mussels from spat collectors were collected from both the Oosterschelde estuary (The Netherlands NL) and Limfjorden (Denmark DK). Upon arrival at the Danish Shellfish Centre (DTU Aqua), mussels were acclimatized at 15 °C with 1 μm UV filtered seawater at 27 PSU. After 1 week, mussels from NL and DK were kept in separate tanks and slowly acclimatized at 15, 20 and 25 °C over a two weeks period. Mussels were classified in four size classes (SC): SC1: 10–15 mm, SC2: 25–35 mm, SC3: 40–45 mm, SC4: 55–60. Each tank contained 25 individuals from Denmark (5 × SC1, 10 × SC2, 5 × SC3, 5 × SC4) and 16 individuals from The Netherlands (10 × SC2, 3 × SC3, 3 × SC4). For comparison of the data with the Netherland setup, only data from SC2 are presented in Table 3 and in all the graphs and analysis. A flow-through system was designed to supply nine water treatments (Fig. 1b) to the mussels with three temperatures (15, 20 and 25 °C) each at three levels of oxygen saturation (100, 50 and 30%). Oxygen saturation levels were measured with OxyGuard Commander probes and controlled using the OxyGuard Pacific (OxyGuard International A/S, Birkerød, Denmark) including a relay with solenoid valves that regulated nitrogen gas delivery to wooden air stones to the nine 200 L tanks, while the 100% saturation was maintained by air bubbling. The system temperature was set at 15 °C and warmed up to 20 and 25 °C with water heaters. Each tank was equipped with a small water pump to insure mixing of the water. Treated waters were distributed to their respective treatments by gravitation at a flow rate of 10l/h. Each tank had a food supply using pumps. Food constituted of *Rhodomonas salina* cultured indoor with UV filtered water at 0.2 μm. Preliminary clearance rate experiments indicated that mussels from the Netherlands were not clearing *T-isochrysis*, but were actively filtering like Danish mussels *Rhodomonas salina*. Thus, *Rhodomonas* was selected. The daily amount was calculated based on the concentration of the culture and the flow rate in each tanks. The high food treatment (>8 μg/L) was four time more concentrated than the low food treatment (2 μg/L). Food containers of 200L were replenished 1–3 times a day. A pump was recirculating the food within the feeding tanks and drops of food were constantly dripping in each experimental tank. Food concentration in each tanks was measured several time a day to adjust food flow rate. Experiment lasted between 21 and 28 days for logistic reasons and morphometric measurements, due to the large number of mussels used for the experiment (1674), the start and end of experiment for each tanks could not be on the same day.

2.2 Monitoring environmental conditions during the experiments

Chlorophyll concentrations in the tanks of the Temp/Food Experiment were measured every 10 min for a period of

Fig. 1. Experimental design with different temperature, food and oxygen levels. a. Basins (coloured rectangles) with three replicate tanks (open black circles) containing five suspended nets (solid black circles) with ten individuals per species and origin (Dutch Mytilus edulis, Danish *Mytilus edulis*, Portugese *Mytilus galloprovincialis*, Dutch *Crassostrea gigas* and Dutch *Ostrea edulis*). b. Replicate tanks (black circles) containing 41 individually marked mussels Mytilus edulis from Dutch and Danish origin (black and grey ellipses). Main supply of water with aeration or N_2 providing three different O_2 saturation levels to the different replicates.

24 hours on 15 days spread over the experiment using an Infinity fluorometer (JFE Advantech Co, Japan). In addition, temperature was monitored every 30 minutes with i-buttons in each basin holding the tanks. Parameters indicative of water quality were monitored three times a week just before refreshing of the water: salinity, O_2 concentration and pH were measured using a Hach HQd Field case Cat. No. 58357-000. The NO_2 and NH_4 concentrations were determined once using TETRA Tests. In the Temp/Food/Ox, experiment temperature and oxygen saturation were measured at an interval of 10 min in the water supply tanks and three experimental tanks. Chlorophyll *a*, temperature and oxygen were measured two to three times a day in all the 54 tanks using a Turner 10AU and an hand held OxyGuard. Inflow salinity was measured daily. Other water quality parameters such as pH, NO_2 and NH_4 concentrations were not measured, as it was assumed that the renewal of 10% water per day in the flow-through system of each tank was enough to maintain optimal conditions.

2.3 Bivalve survival, growth and condition

After the acclimation period in the set-up of the Temp/Food Experiment, ten individuals per species and per net were

Table 2. Initial shell length (mm) with standard deviation (sd, $n = 30$) of bivalves used in the temp/food experiment.

Treatment	*Mytilus edulis* NL	*Mytilus edulis* DK	*Mytilus galloprovincialis*	*Crassostra gigas*	*Ostrea edulis*
3 °C, 2 µg	27.22 ± 2.71	28.79 ± 1.36	25.66 ± 1.68	19.98 ± 2.66	23.00 ± 2.76
3 °C, 10 µg	29.04 ± 1.91	29.07 ± 1.10	25.82 ± 1.77	20.37 ± 2.49	21.36 ± 2.84
8 °C, 2 µg	29.90 ± 1.90	28.39 ± 0.95	25.58 ± 1.22	18.92 ± 1.96	23.40 ± 3.20
8 °C, 10 µg	29.58 ± 2.34	28.35 ± 0.83	25.97 ± 1.46	20.54 ± 3.20	22.11 ± 2.92
15 °C, 2 µg	27.98 ± 2.09	28.08 ± 1.25	25.84 ± 1.38	21.12 ± 3.14	22.48 ± 2.82
15 °C, 10 µg	29.17 ± 2.22	29.46 ± 1.33	24.61 ± 1.54	19.47 ± 2.34	21.86 ± 3.71
20 °C, 2 µg	31.15 ± 1.81	29.44 ± 1.26	25.13 ± 1.70	20.63 ± 2.49	24.01 ± 2.92
20 °C, 10 µg	30.43 ± 2.52	27.79 ± 1.50	26.45 ± 2.01	21.79 ± 2.60	23.53 ± 2.60
25 °C, 2 µg	29.94 ± 1.94	28.91 ± 1.05	25.35 ± 1.27	21.10 ± 2.53	23.07 ± 2.19
25 °C, 10 µg	29.52 ± 2.21	28.56 ± 1.47	25.61 ± 2.39	19.61 ± 2.13	23.61 ± 2.51
30 °C, 2 µg	30.09 ± 2.71	29.55 ± 1.16	25.74 ± 2.76	20.89 ± 2.18	22.00 ± 2.27
30 °C, 10 µg	30.90 ± 2.37	27.94 ± 1.42	25.04 ± 1.61	21.23 ± 3.42	21.99 ± 3.12

Table 3. Initial shell length (mm) with standard deviation (sd, $n = 30$) of mussel *Mytilus edulis* used in the Temp/Food/Ox Experiment for size class 2.

Treatment	*Mytilus edulis* DK	*Mytilus edulis* NL
15 °C, 2 µg, 100%	30.31 ± 3.03	30.77 ± 3.43
15 °C, 2 µg, 50%	30.60 ± 2.68	30.30 ± 3.00
15 °C, 2 µg, 30%	30.82 ± 3.15	30.46 ± 2.46
15 °C, > 8 µg, 100%	30.75 ± 3.08	30.11 ± 2.85
15 °C, > 8 µg, 50%	30.02 ± 3.00	30.40 ± 3.30
15 °C, > 8 µg, 30%	29.93 ± 3.45	30.83 ± 2.70
20 °C, 2 µg, 100%	31.14 ± 3.06	31.19 ± 2.27
20 °C, 2 µg, 50%	30.46 ± 3.23	30.95 ± 2.60
20 °C, 2 µg, 30%	30.63 ± 2.87	30.92 ± 3.04
20 °C, > 8 µg, 100%	30.42 ± 3.23	30.42 ± 2.66
20 °C, > 8 µg, 50%	30.56 ± 3.10	30.60 ± 2.42
20 °C, > 8 µg, 30%	30.30 ± 2.91	30.05 ± 2.67
25 °C, 2 µg, 100%	30.91 ± 3.18	30.46 ± 2.68
25 °C, 2 µg, 50%	31.00 ± 2.71	31.02 ± 2.78
25 °C, 2 µg, 30%	30.64 ± 3.07	30.62 ± 2.57
25 °C, > 8 µg, 100%	29.07 ± 2.82	29.58 ± 2.58
25 °C, > 8 µg, 50%	30.23 ± 3.03	30.54 ± 2.97
25 °C, > 8 µg, 30%	30.47 ± 2.75	30.01 ± 3.05

selected randomly and measured for initial shell length (SL mm) with digital calipers and wet weight (WWt g) with a balance. The individuals were individually marked by using nail polish with different colours and placed in small nets per species and origin. Live animals were counted in each net three times a week, and dead specimens were removed. During acclimation period of the Temp/Food/Ox Experiment, all mussels were measured for initial SL (mm) and WWt (g). All mussels were individually tagged using bee-tags glued with Dupla plantfix cyanoacrylate. Each tanks were inspected daily and dead mussels removed.

At the end of the Temp/Food Experiment, after 6 weeks, shell length and wet weight of the surviving individuals was measured again. Growth rates was calculated as as increase in wet weight and in shell length according to $((\mathrm{LN}\, W_f - \mathrm{LN}\, W_i)/t) * 100$, where W_f = final wet weight in g; W_i = initial wet weight in g; t = time in days and to $L_{\mathrm{end}} - L_{\mathrm{begin}}$/time, where L_{end} = final shell length in mm; L_{begin} = initial shell length in mm; t = time in days. In addition, ash-free dry weight (AFDW) of the bivalves was determined per net with a prep ash by first measuring the dry weight (DW) of the flesh and shell separately after drying at 70 °C to constant weight, followed by measuring ash-weight (AW) of the flesh by ashing at 540 °C. The DW and AW were used to calculate the AFDW by subtracting AW from DW (DW–AW) and Condition Index (CI) according to (AFDW meat/DW shell) *100. At the end of the Temp/Food/Ox Experiment, after 21–28 days, both SL and WWt were measured. All animals were dissected, shell weight, flesh and gonad wet, dry and ash free dry weight were measured as well as gill areas. AW, DW,

AFDW and CI were measured and calculated as described above.

2.4 Bivalve clearance rate and oxygen consumption

Clearance rate (CR) was measured at the end of the Temp/Food Experiment. CR is the rate at which a certain volume of water is cleared from all particles. For this the particles need to be 100% efficiently retained by the bivalve gills (Smaal, 1997). The algal species we used were in the size range 4–10 μm, which are 100% efficiently retained (Cranford *et al.*, 2011). For each treatment, clearance rates were measured in an experimental flow-through system. Specimens of a net were placed in 100 ml containers through which water was pumped with a peristaltic pump from the tank in which they were kept during the growth experiment. Flow rates were adjusted such that a significant difference in particle concentration (approx. 30%) was detected between the water flowing into the containers and the water flowing out of the containers (Pascoe *et al.*, 2009). Flow rates varied between 2 and 3 l/h. The bivalves were given time to adapt (approx. 1 h), after which the clearance rates were measured. For this, samples from the outflowing water were sampled for each container and the concentration of particles ranging between 4 and 10 μm was analysed using an Accuri Flow Cytometer. To correct for particle settlement inside the containers we measured the particle concentration in the water flowing out of a control treatment, a container without bivalves. The CR was determined by measuring the number of particles in the outflow and comparing these data with the outflow of the control. For the calculation of the CR the following formula (Widdows, 1985) was used:

$$CR = ((C_{in} - C_{out})/C_{in}) * Q$$

where CR = clearance rate in l/h per individual; C_{in} = particle concentration of the outflow of the control (number/l); C_{out} = particle concentration of the outflow (number/l); Q = flow rate in l/h.

The CR was then adjusted to l/h/g DW based on the DW determinations of each net.

CR were weight standardized (Bayne et al., 1987) using the following equation:

$$CRs = CR * (1/DW)^b$$

where $b = 0.67$ according to Møhlenberg and Riisgård (1979).

In the Temp/Food/Ox Experiment CR were measured during the experiment were selected mussels were removed from the tanks. CR were measured using the indirect clearance method (Riisgård, 2001) where the reduction in number of particles over time is monitored by taking samples at fixed time interval.

$$CR = (V/nt)\ln(C_0/C_t)$$

where CR = clearance rate (l/h), C_0 and C_t = algal concentration (raw fluorescence) at time 0 and time t, V = volume of water (l), n = number of individual, t = time (h).

CR were weight standardized as described above.

At least four clearance were run per experiment cycle. The first run was discarded and considered as acclimation to the setup. Mussels were placed individually in sealed plastic jars connected with 3 tubing on the lid in order to keep the SO_2 (oxygen saturation) according to the treatments and avoid any gas exchange. Two were used for in and out flow of the jar in a closed circuit connected to a peristaltic pump for mixing of the water without gas exchange. The third tube was used to take in vivo chlorophyll a sample with a 50 mL syringe, which once read was returned to the jar using a 5 ml pipette. A set of 10 sealed jars were placed in a Bain Marie to keep the temperature stable. Eight jars were used for mussels from both DK and NL, while two jars were used as control. SO_2 was measured before and after the experiment to insure constant conditions.

In the Temp/Food/Ox Experiment respiration rates (RR) were measured on individual mussels using four respiratory chambers (138 ml) fitted with flushing tubes and oxygen spots connected to an Oxy-4-mini system (Loligo System; Viborg, Denmark). Chambers were immersed in sealed tank filed with filtered seawater, temperature was controlled by heater, while SO_2 was maintained at 100% using air stones and at 50 or 30% using wooden air stone with N_2 gas. Mixing of the chambers was insured by magnetic stirrers. Mussels were deposited on top of a small solid mesh to avoid contact with the sitter. A mini 5W aquarium pump (EHEIM, Hmax 0.5m) was fitted to the four chambers and were flushed when activated at known intervals using Arduino system (open electronic platform). The flushing and reading cycles were set by default to 6 and 10 minutes respectively and adapted depending on mussel respiration rates. Oxygen probes were connected to a OXY-4 mini four-channel fiber-optic oxygen transmitter (PreSens, Regensburg, Germany). Oxygen values were recorded via OXY4v2_11FB software (Loligo Systems, Viborg, Denmark). At least 3 mussels per treatment were used for the respiration rates measurements. Only the full set of mussel size 2–3 at low food concentration were measured (17 sets of experiment on 51 mussels), while selected treatments were measured for the high food concentration (5 sets of experiment on 15 mussels). Starved mussels of different size classes were also used to measure respiration rates (24 sets of experiments on 71 mussels).

Oxygen consumption was calculated using the mussel biomass, respiratory chamber volume. Oxygen concentration in the chamber was measured every 5 seconds and plotted as hPa in function of time and a linear regression was added. Respiration rate (RR, mg O_2 /min) was calculated as follow:

$$RR = (b/a) \times V_{cor}$$

where b is the slope of the regression line, a is the solubility mgO_2/L/hPa calculated from Loligo website (https://www.loligosystems.com/convert-oxygen-units) varying in function of salinity, temperature and pressure, and V_{cor} is the volume of the chamber minus the volume of the mussel. Respiration rates were corrected by substracting the potential oxygen consumption present in the control chambers.

RR were weight standardized (Bayne et al., 1987) using the following equation:

$$RRs = RR * (1/DW)^b$$

where $b = 0.75$ according to Bayne and Newell (1983).

Table 4. Average conditions (with sd) in tanks during the Temp/Food Experiment.

Treatment	Temperature (°C) (°C)	Chlorophyll *a* (μg/l) (μg/l)	Salinity	O_2 (mg/l) (mg/l)	pH	NO_2 (mg/l)	NH_4 (mg/l)
3 °C, 2 μg	3.3 ± 1.6	2.62 ± 1.60	33.2 ± 0.4	12.05 ± 0.29	8.09 ± 0.31	0.025	0.000
3 °C, 10 μg	3.8 ± 2.0	12.82 ± 13.29	33.4 ± 0.3	12.07 ± 0.56	8.08 ± 0.30	0.025	0.000
8 °C, 2 μg	8.4 ± 0.2	2.58 ± 0.70	33.1 ± 0.2	10.62 ± 0.27	8.08 ± 0.30	0.050	0.000
8 °C, 10 μg	8.4 ± 0.3	8.87 ± 4.51	33.3 ± 0.3	10.46 ± 0.34	8.05 ± 0.30	0.075	0.000
15 °C, 2 μg	15.2 ± 0.5	2.90 ± 0.70	32.5 ± 0.2	9.02 ± 0.24	8.03 ± 0.32	0.000	0.000
15 °C, 10 μg	16.7 ± 0.4	7.52 ± 1.87	32.7 ± 0.3	8.94 ± 0.25	8.00 ± 0.33	0.400	0.000
20 °C, 2 μg	20.0 ± 0.4	2.71 ± 0.52	32.4 ± 0.3	8.47 ± 0.27	8.09 ± 0.28	0.300	0.000
20 °C, 10 μg	20.6 ± 0.9	8.42 ± 3.67	32.8 ± 0.2	8.28 ± 0.15	7.97 ± 0.35	0.500	0.000
25 °C, 2 μg	25.0 ± 0.7	2.92 ± 1.06	33.6 ± 0.4	7.80 ± 0.12	8.13 ± 0.28	0.500	0.250
25 °C, 10 μg	26.2 ± 0.6	6.99 ± 2.99	33.8 ± 0.5	7.80 ± 0.20	7.99 ± 0.36	0.500	0.000
30 °C, 2 μg	30.7 ± 0.9	3.46 ± 1.38	34.5 ± 0.9	7.57 ± 0.19	8.10 ± 0.34	0.100	0.000
30 °C, 10 μg	30.9 ± 0.8	7.56 ± 3.36	34.9 ± 0.5	7.42 ± 0.18	8.03 ± 0.36	0.300	0.200

2.5 Statistical analysis

Differences between treatments and effects of food, temperature and oxygen levels on survival duration, growth rates, clearance rates and oxygen consumption rates were tested with Analysis of Variance (ANOVA). Linearity of the data was examined with residual plots. The homogeneity of variances was tested with a Bonferroni or Levene tests. When the variances were not distributed homogeneously, the data were transformed (square root for counts and $1/(x+1)$ for rates). When ANOVA assumptions were still violated, a non-parametric Mann–Whitney test was performed. Significant effects between shellfish of different origins were examined using posthoc Bonferroni tests. Statistical analyses were performed using IBM SPSS 25 and JMP ® Pro 15.0.0.

3 Results

3.1 Environmental conditions during the experiments

3.1.1 Temp/food experiment

The temperature and chlorophyll concentrations in the tanks were kept at the desired level, except for the slightly higher chlorophyll concentration of 12 μg/l at 3 °C (Tab. 4). The other parameters (pH 8, O_2 concentration >7.7 mg/l, salinity 33–34, NO_2 and NH_4 concentrations ≤0.5 mg/l) were optimal for shellfish (Blanco Garcia and Kamermans, 2015). Salinity increased with increasing temperature, possibly due to evaporation of the water (Tab. 4). Oxygen concentration decreased with increasing temperature, as warmer water holds less dissolved oxygen.

3.1.2 Temp/food/ox experiment

The temperature and oxygen saturation were kept at the desired levels, except for the temperature category of 25 °C, were the temperature was lower in average 22.9 ± 1.4 °C (Tab. 5). Salinity was in average 27.34 ± 1.06 during the experimental period. Chlorophyll *a* in the tanks was fluctuating (Tab. 5). However, the same amount with same quality of food was added to the tanks either in Low or High category. In order to ascertain that High food regime was always superior than Low food regime, the concentration was always 4 time more concentrated.

3.2 Bivalve survival, growth and condition

3.2.1 Temp/food experiment

Survival of the bivalves during the experiment ranged from 93% to 100% except for the mussels exposed to 30 °C. In those treatments all mussels died (Fig. 2). After three to five days all *M. edulis* from The Netherlands (NL) and Denmark (DK) had died. The *M. galloprovincialis* from Portugal (PT) lasted significantly longer (Supplementary Material Tab. S.1): 16 days at low food concentrations and 32 days at high food concentrations. In the Temp/Food/Ox Experiment, the survival rate was 99.46% with only 12 mussels out of 2214 dying during the experiment. Mortality did not follow a particular treatment pattern as all dead mussels originated from across the size range and random treatments.

A significant effect of food on growth was observed (Figs. 3a & 4a and Tab. S.2). At low food concentration maximal shell growth rate for mussels was 0.0283 ± 0.0060 mm day^{-1}, while it was 0.1703 ± 0.0297 mm day^{-1} at high food concentration. At low food concentration maximal specific growth rate for mussels was 0.2651 ± 0.0191, while it was 1.7697 ± 0.2177 at high food concentration. For oysters maximal shell growth rate was 0.0616 ± 0.0115 mm day^{-1} at low food concentration, while it was 0.2548 ± 0.0279 mm day^{-1} at high food concentrations. Maximal specific growth rate of oysters was 0.5608 ± 0.0856 at low food concentration, while it was 2.2855 ± 0.2169 at high food concentrations. The growth results showed that effects of temperature were affected by significant interactions with food. Higher food conditions showed higher optimal temperatures for growth of mussels and oysters.

Condition of the shellfish was significantly affected by temperature and food and their interaction (Fig. 5a and Tab. S.4). Condition index was higher at higher food concentrations and decreased with increasing temperature. In addition, Pacific oysters had a significantly higher condition index than flat oysters (Fig. 5a, Tab. S.4).

Table 5. Average conditions (with sd) in tanks during the Temp/Food/Ox Experiment.

Treatment	Temperature (°C)	Chlorophyll *a* (µg/l)	O_2 (%)	O_2 (mg/l)
15 °C, > 8 µg, 100%	15.9 ± 0.8	3.91 ± 3.25	91.8 ± 5.6	7.7 ± 0.5
20 °C, > 8 µg, 100%	19.9 ± 1.4	4.58 ± 4.92	80.1 ± 10.4	6.2 ± 0.9
25 °C, > 8 µg, 100%	22.6 ± 1.5	5.04 ± 5.01	81.9 ± 6.9	6.1 ± 0.4
15 °C, > 8 µg, 30%	16.6 ± 0.8	8.57 ± 18.79	27.4 ± 8.0	2.3 ± 0.7
20 °C, > 8 µg, 30%	20.4 ± 1.7	6.08 ± 8.76	27.0 ± 8.2	2.1 ± 0.7
25 °C, > 8 µg, 30%	23.7 ± 1.1	10.16 ± 17.24	26.4 ± 7.6	1.9 ± 0.6
15 °C, > 8 µg, 50%	16.3 ± 0.8	5.29 ± 7.52	41.9 ± 8.8	3.9 ± 1.7
20 °C, > 8 µg, 50%	20.3 ± 1.5	5.53 ± 6.14	42.8 ± 9.1	3.3 ± 0.8
25 °C, > 8 µg, 50%	23.3 ± 1.6	7.07 ± 7.87	42.0 ± 8.0	3.1 ± 0.6
15 °C, 2 µg, 100%	15.9 ± 1.0	2.20 ± 4.57	92.6 ± 14.8	8.4 ± 0.5
20 °C, 2 µg, 100%	19.8 ± 1.8	2.43 ± 2.48	92.6 ± 3.5	7.2 ± 0.4
25 °C, 2 µg, 100%	22.1 ± 1.2	2.47 ± 3.17	87.7 ± 7.6	6.5 ± 0.5
15 °C, 2 µg, 30%	15.5 ± 0.9	1.64 ± 2.03	35.5 ± 4.9	3.0 ± 0.4
20 °C, 2 µg, 30%	20.3 ± 1.4	2.90 ± 3.36	28.4 ± 8.8	2.2 ± 0.7
25 °C, 2 µg, 30%	23.2 ± 1.3	3.65 ± 4.00	21.8 ± 10.6	1.7 ± 0.8
15 °C, 2 µg, 50%	16.6 ± 0.8	1.96 ± 2.16	52.2 ± 6.2	4.7 ± 1.2
20 °C, 2 µg, 50%	20.2 ± 1.3	2.04 ± 1.66	49.7 ± 4.5	3.8 ± 0.4
25 °C, 2 µg, 50%	22.9 ± 1.3	2.34 ± 1.71	49.3 ± 18.6	3.6 ± 1.4

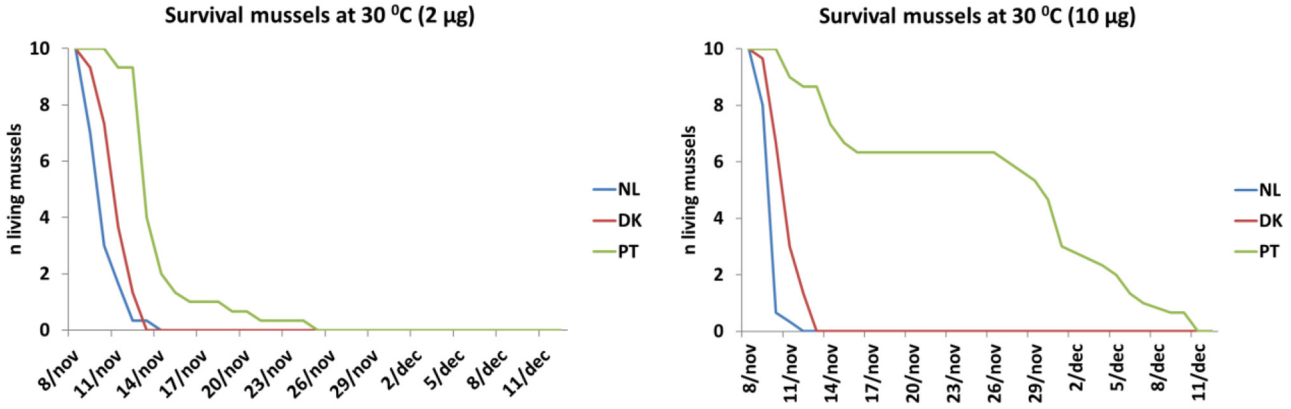

Fig. 2. Survival of *M. edulis* from Denmark (DK) and The Netherlands (NL) and *M. galloprovincialis* from Portugal (PT) at 30 °C.

3.2.2 Temp/food/Ox experiment

Mussel growth was found to be significantly affected by oxygen concentration. At the high food treatment, reduced O_2 concentrations resulted in lower growth, the shell growth rate (mm day^{-1}) was 0.079 ± 0.0.036 for DK *M. edulis* at 15 °C 100% O_2 saturation vs 0.057 ± 0.028 at 30% O_2 saturation and this was exacerbated at higher temperature: 25 °C 100% O_2 0.070 ± 0.033 vs 25 °C 30% O_2 0.016 ± 0.012 . At the low food treatment there were no differences in growth among the different O_2 concentrations at the same temperature (Figs. 3b and 4b and Tab. S.3).

The NL mussels had a significantly lower length growth rate than the DK mussels for the size class 2–3 cm (Mann–Whitney test $W = 356309.0$, $p < 0.001$) and significantly lower growth at low food concentration (Mann–Whitney test $W = 83982.5$, $p < 0.001$) as many mussels did not grow at low food concentration and low temperature. This was also visible for the specific growth rate (Tab. S.3 and Fig. 3b). As a result, at low food concentration, the only significant factor was low temperature, where at 15 °C both DK and NL mussels grew more than for all other temperature and oxygen saturation (Tab. S.3, Figs. 3b and 4b). The specific growth rate was 0.87 ± 0.6 for DK *M. edulis* at 15 °C 100% O_2 saturation vs 0.61 ± 0.36 at 30% O_2 saturation and the difference was increased at higher temperature: 25 °C 100% O_2 0.82 ± 0.33 vs 25 °C 30% O_2 0.36 ± 0.38. At high food concentration treatment, both DK and NL mussels growth was higher with low temperature and high oxygen level, the interaction of high temperature and low oxygen saturation reduced growth (Fig. 4b).

DK CI was superior to NL (Tab. S.3). CI was lower at low oxygen saturation (Fig. 5b, Tab. S.3).

3.3 Bivalve clearance rate and oxygen consumption

3.3.1 Temp/food experiment

A significant effect of temperature and food concentration on clearance rate was found (Fig. 6a and Tab. S.5). Lower clearance rates were observed at high food concentrations.

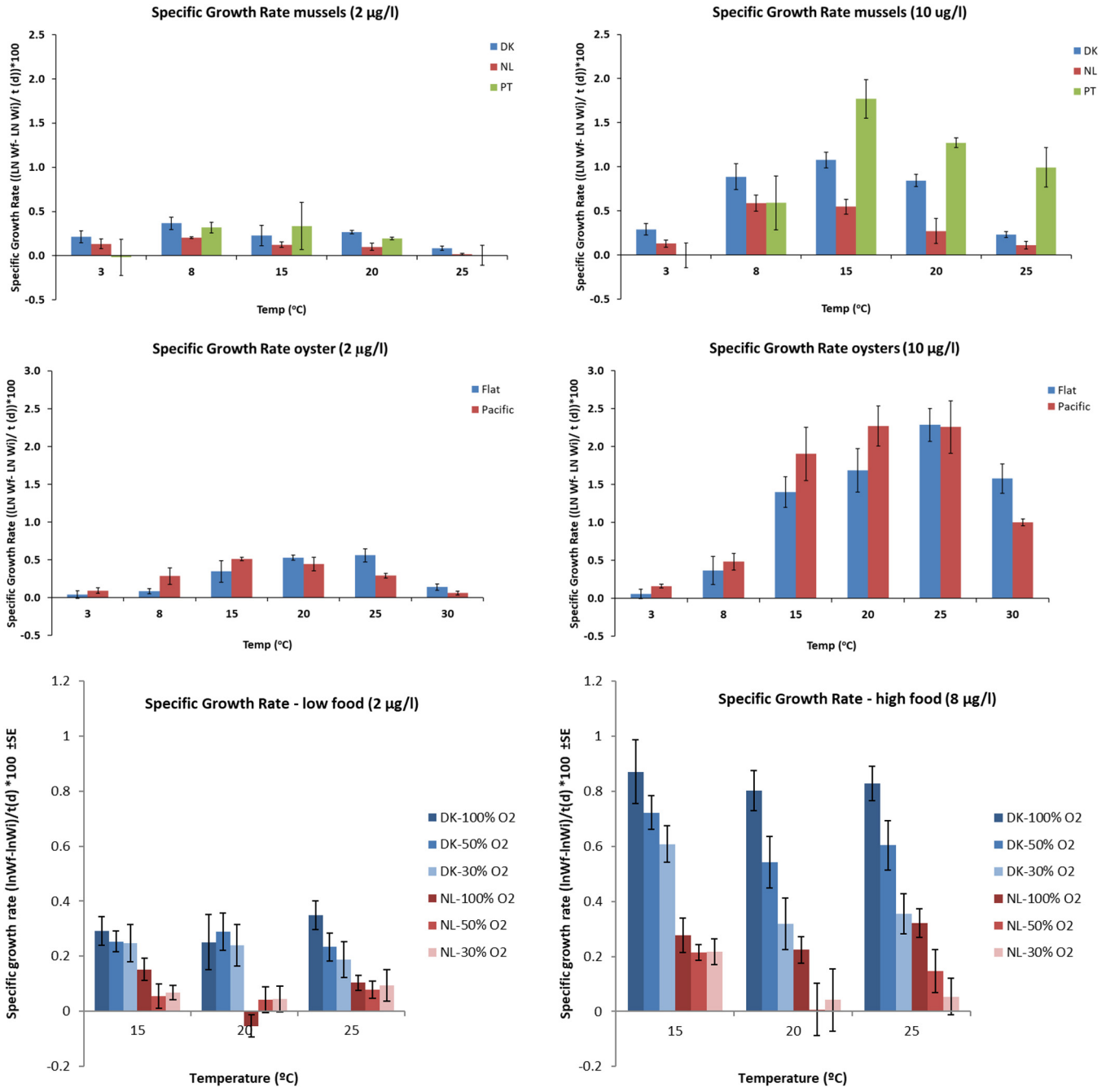

Fig. 3. (a) Growth rates based on wet weight of *M. edulis* from Denmark (DK) and The Netherlands (NL), *M. galloprovincialis* from Portugal (PT), *O. edulis* (Flat) and *C. gigas* (Pacific) from The Netherlands exposed to different temperatures and food concentrations for 6 weeks. (b) Growth rates based on wet weight of *M. edulis* from Denmark (DK) and The Netherlands (NL), from Denmark exposed to different temperatures (15, 20 and 25 °C), oxygen saturation (30, 50 and 100%) and food concentrations (2 and 8 µg/L) for 21 to 28 days experiment.

3.3.2 Temp/food/ox experiment

In this experiment oxygen saturation was added as a treatment and clearance rates were significantly reduced with low oxygen concentrations combined with high temperature (Fig. 6b and Tab. S.6). Average food concentration in the experimental chambers for the CR experiment varied between 1.6 and 10.2 µg/L. In order to remove potential overlapping of food values for the two categories of food treatment, CR measurements carried out at maximum concentrations between 4 and 6 µg/L were removed from the analysis. With the reclassification, all factors excepting the origin were significantly affecting the CR. Cross factors including origin where removed from the model (Tab. S.6, Fig. 6b). Maximum CR was at 15 °C and minimum at low oxygen concentration. Food was significant when oxygen conditions were not 100%. At 100% saturation of oxygen, clearance rate increased with temperature at High food regime, but not at Low food regime.

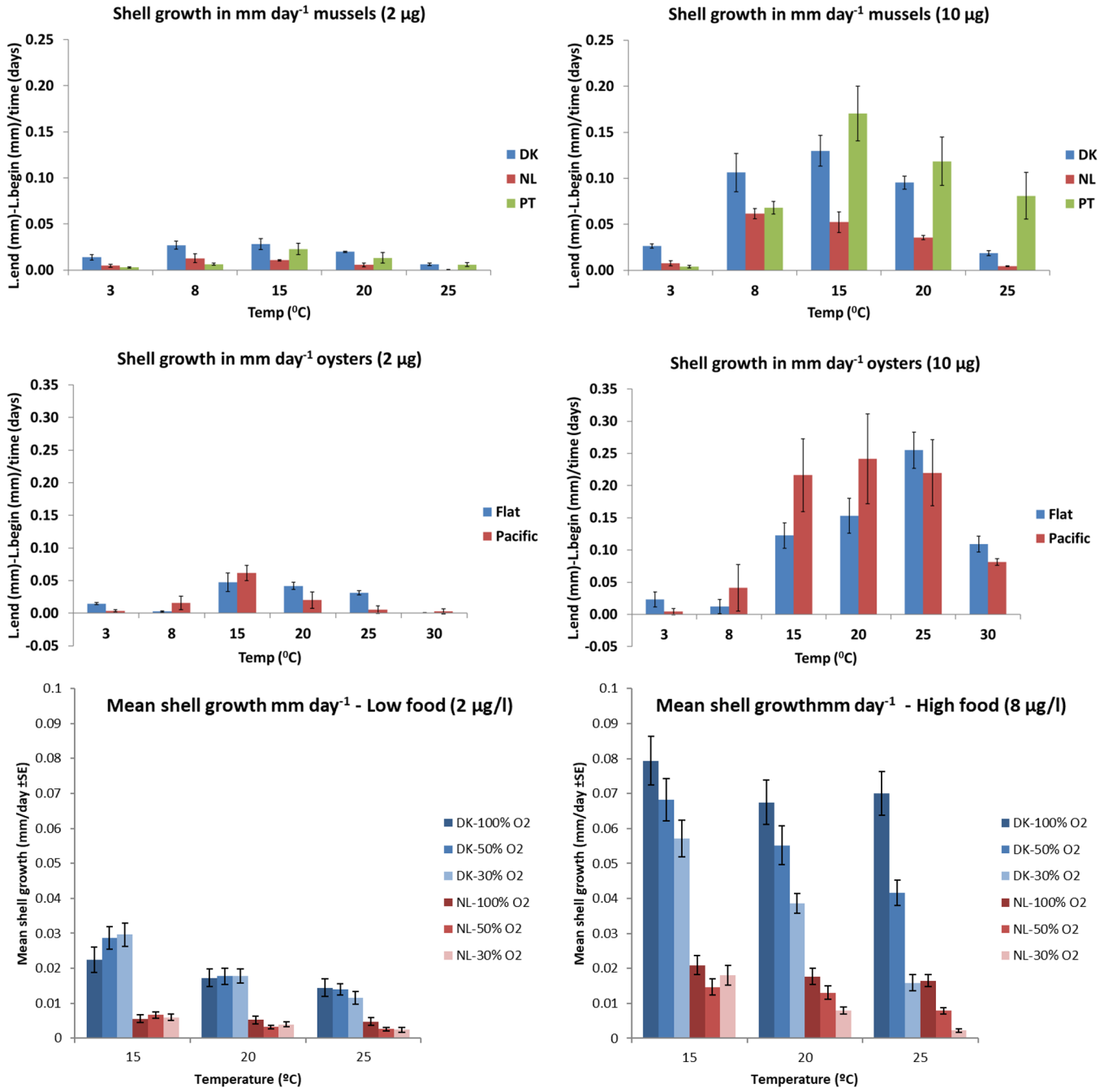

Fig. 4. (a) Growth rates based on shell size of *M. edulis* from Denmark (DK) and The Netherlands (NL), *M. galloprovincialis* from Portugal (PT), *O. edulis* (Flat) and *C. gigas* (Pacific) from The Netherlands exposed to different temperatures and food concentrations for 6 weeks. (b) Growth rates based on shell size of *M. edulis* from Denmark (DK) and The Netherlands (NL), from Denmark exposed to different temperatures (15, 20 and 25 °C), Oxygen saturation (30, 50 and 100%) and food concentrations (2 and 8 µg/L) for 21 to 28 days experiment.

Oxygen saturation was the main factor affecting the CR. High temperature and low oxygen concentration combined, significantly reduced clearance rate and increased oxygen consumption (Tab. S.6 and Fig. 6b). Respiration rates at Low oxygen saturation at 25 °C for NL mussels were significantly higher than all respiration rates for both species 15 and 20 °C and all SO_2 at low food concentration (Tab. S.7 and Fig. 7). On the other hand, at 20 °C and low SO2, RR for NL mussels were significantly lower than DK RR at 25 °C. Overall the RR were not significantly different at low or high food concentration with increase of temperature (Tab. S.7 and Fig. 7).

4 Discussion

The combination of controlled environmental conditions in a factorial design in order to model the impact of their interaction on the physiological responses of bivalves is

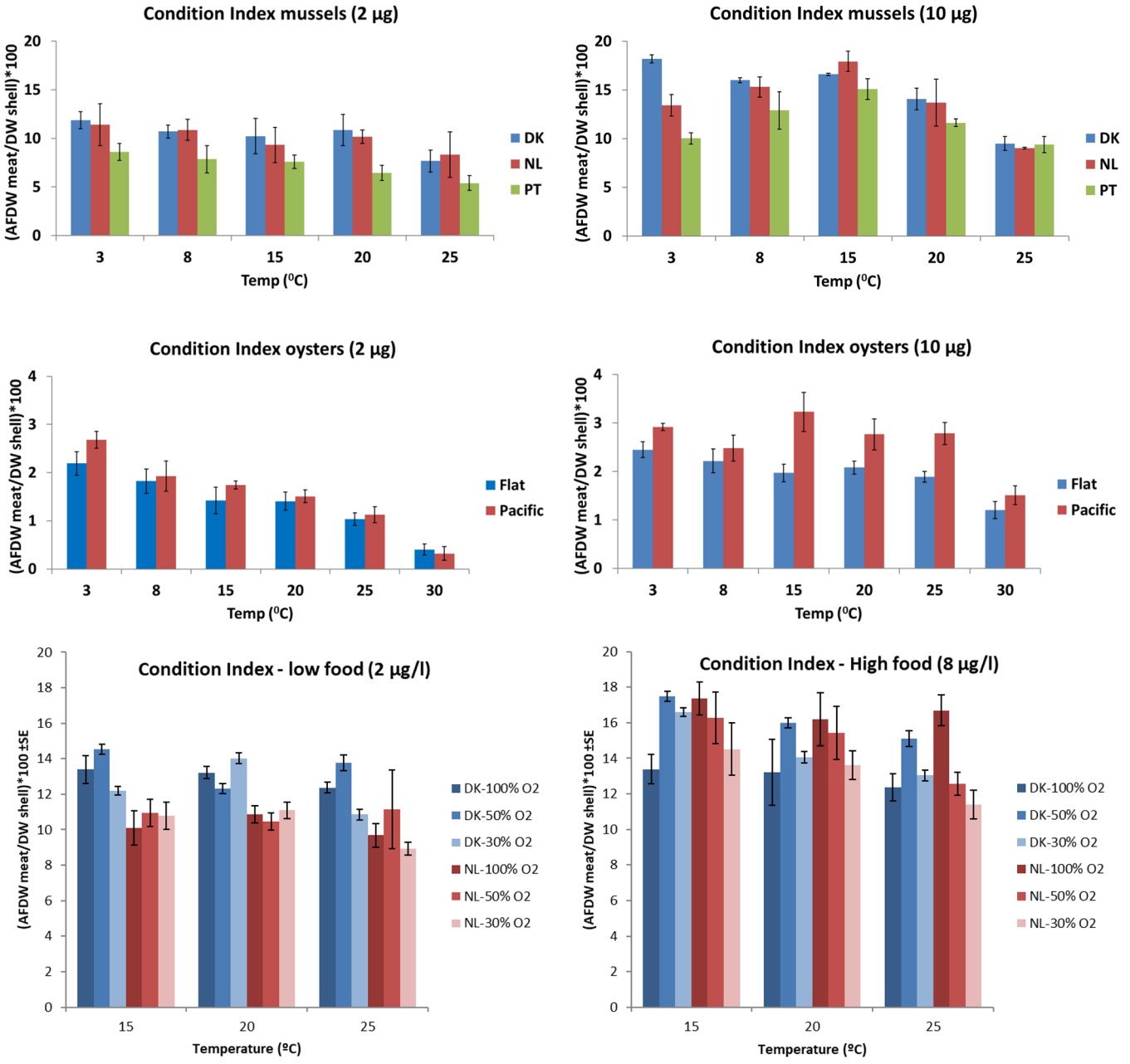

Fig. 5. (a) Condition of *M. edulis* from Denmark (DK) and The Netherlands (NL), *M. galloprovincialis* from Portugal (PT), *O. edulis* (Flat) and *C. gigas* (Pacific) from The Netherlands exposed to different temperatures and food concentrations for 6 weeks. (b) Condition of *M. edulis* from Denmark (DK) and The Netherlands (NL) from Denmark exposed to different temperatures (15, 20 and 25 °C), Oxygen saturation (30, 50 and 100%) and food concentrations (2 and 8 µg/L) for 21 to 28 days experiment.

technically challenging. However, it is important to acquire in order to mitigate potential loss in production to the aquaculture and fishery sector development under climate change pressures. This study compiled for the first time the effects of combined environmental treatments on physiological rates of bivalves and documented that for instance the combination of high temperature and low oxygen saturation was enhancing a decrease in clearance rates and growth in blue mussels.

4.1 Survival

The experiments showed that mussels can survive in the temperature range of 3–25 °C, but do not survive at 30 °C. Experiments of Jones *et al.* (2009) showed a similar result for *M. edulis* juveniles on the East coast of the US; mortality increased dramatically at all temperatures above 30 °C. Mesas and Tarifeño (2015) also established an upper lethal

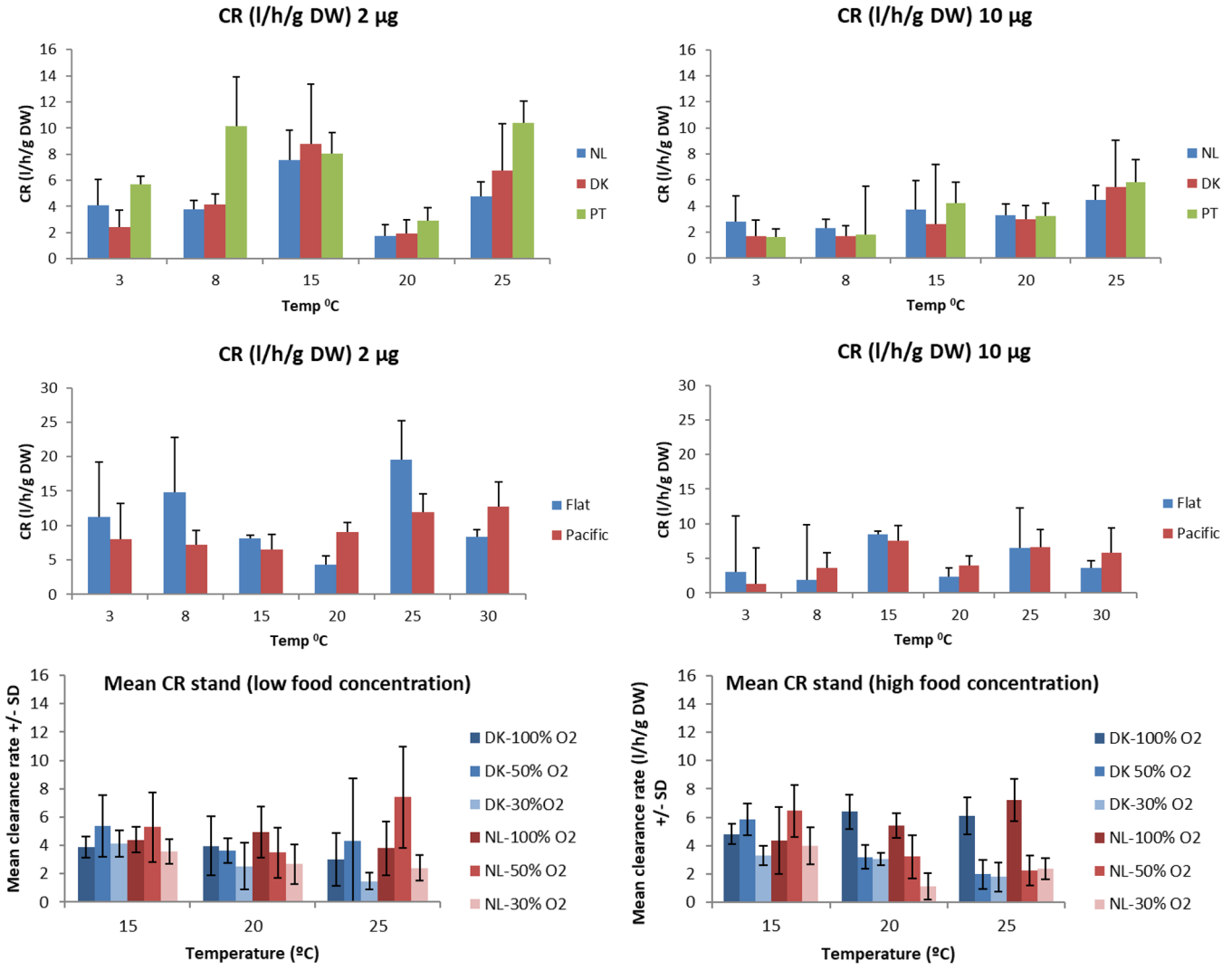

Fig. 6. (a) Clearance rate of *M. edulis* from Denmark (DK) and The Netherlands (NL), *M. galloprovincialis* from Portugal (PT), *O. edulis* (Flat) and *C. gigas* (Pacific) from The Netherlands exposed to different temperatures and food concentrations at the end of a 6-week experiment. (b) Clearance rate of *M. edulis* from Denmark (DK) and The Netherlands (NL), from Denmark exposed to different temperatures (15, 20 and 25 °C), Oxygen saturation (30, 50 and 100%) and food concentrations (2 and 8 µg/L) for 21 to 28 days experiment.

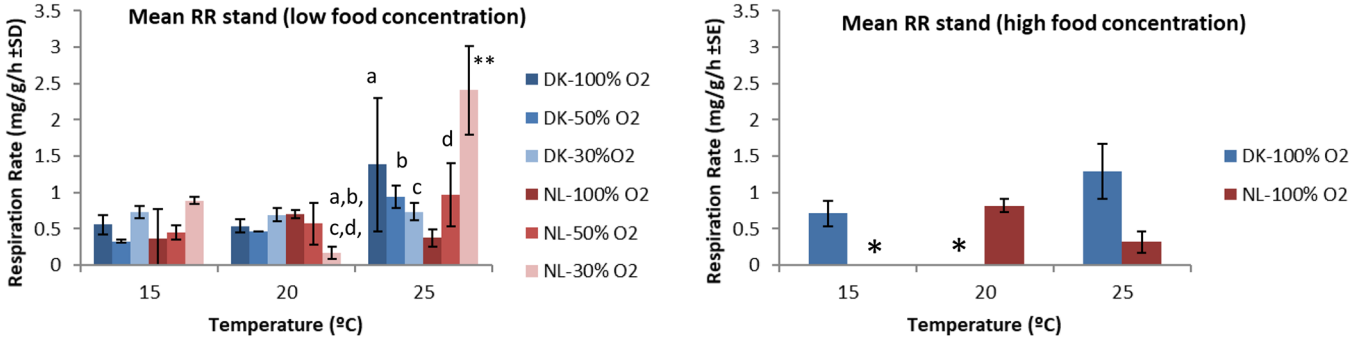

Fig. 7. Oxygen consumption of *M. edulis* from Denmark (DK) and The Netherlands (NL), from Denmark exposed to different temperatures (15, 20 and 25 °C), Oxygen saturation (30, 50 and 100%) and food concentrations (2 and 8 µg/L) for 21 to 28 days experiment. * no data. ** statistically different from all the other treatments, small letters (a to f) indicate statistically different respiration rates. One outlier removed in statistical analysis for DK 100% O_2 25 °C.

temperature of 30 °C for *M. galloprovincialis* adults from Chile in 72 h. Schneider *et al.* (2010) showed a survival of more than 9 weeks for *M. galloprovincialis* juveniles from a US hatchery exposed to 30 °C and better survival under high food conditions. Our study with Portuguese *M. galloprovincialis* juveniles confirmed these findings, but showed a shorter survival time of 4 weeks. Our experiment showed survival of both oyster species at 30 °C. *O. edulis* has been shown to survive at 30 °C (Haure *et al.*, 1998), but not at 36 °C (Eymann *et al.*, 2020). A field study of Castillo-Durán *et al.* (2010) showed some mortality of *C. gigas* in summer, when the water temperature was around 30 °C. This suggests that 30 °C is the upper limit for that species.

The mussels in our experiment survived at 3 °C. *M. edulis* can survive and grow under ice cover (Lauzon-Guay *et al.*, 2006). Jansen *et al.* (2009) determined the minimum temperature for aerobic metabolism in 12 populations along the European coastline and observed a temperature around 4 °C for populations from the Netherlands and Denmark, while the temperature was around 8 °C for a populations of the North of Spain. Portuguese populations were not sampled in this study. The oysters in our experiment showed survival at 3 °C as well. Child and Laign (1998) tested survival of *O. edulis* and *C. gigas* at 3 °C and found survival for *O. edulis*, but *C. gigas* juveniles showed high mortalities after 3–7 weeks. The 100% survival of the *O. edulis* and *C. gigas* juveniles in our 6-week experiment was within this time frame.

4.2 Growth and condition

As expected, growth rate and condition index were higher at higher food concentrations. Higher food conditions showed higher optimal temperatures for growth of mussels and oysters. For the mussels the optimal temperature was 8 °C at low food conditions and 15 °C at high food condition. The optimal temperature for growth of the Pacific oyster was 15 °C at low food conditions and 20 °C at high food conditions and the flat oyster showed optimal growth under low food at 20 °C and under high food at 25 °C. Studies on interactions between these two factors are scarce (Schneider *et al.*, 2010). However, a potential explanation for a shift towards higher optimal temperatures for growth with increase food availability can be that the bivalves can cope better with higher temperatures when provided more food. All bivalves, except *M. galloprovincialis* showed some growth at 3 °C. However, a long-term effect of low temperatures on growth and condition of oysters is unclear. In an 11-week experiment, Child and Laing (1998) observed that *O. edulis* and *C. gigas* juveniles lost weight at 3 °C.

Our experiment showed that *M. edulis* grew less when oxygen concentration was reduced. This effect was more pronounced at higher temperatures, possibly due to increased oxygen demand. Tang and Riisgård (2018) observed that *M. edulis* is no longer able to maintain its normal aerobic metabolism at oxygen concentrations below 2 mg O_2/L (which is just below our 30% treatment), and attribute this to an energy-saving mechanism to strongly reduce the activity when exposed to low oxygen conditions.

A remarkable results is that *M. edulis* from the Netherlands showed lower growth rates and shell growth than *M. edulis* from Denmark in both experiments, independent of the environmental conditions. A potential bias due to the existence of different breeding paths cannot be ruled out. In August 2016, the mussels collected in the Oosterschelde estuary contained granulocytoma's (Capelle *et al.*, 2021). This inflammation causes lesions in the digestive glad and mantle tissues and is generally considered an indicator of chronic stress (Lowe and Moore, 1979). The Dutch mussels used in the experiments were collected in the Oosterschelde estuary in October 2016. Potentially granulocytoma's were still present and this may explain their lower growth rate.

4.3 Clearance rate and oxygen consumption

Widdows (1976) showed that filtration rate of *M. edulis* acclimated to 11, 15 and 19 °C adapted by reducing the temperature-dependence of filtration rates resulting in a steady-state value which was independent of temperature. Eymann *et al.* (2020) measured filtration rates of *O. edulis* which increased from 3.0 L h^{-1} gDW^{-1} at 14 °C to a maximum of 7.7 L h^{-1} g DW^{-1} at 22 °C followed by a progressive decrease resulting in a minimum 0.3 L h^{-1} g DW^{-1} at 34 °C. We did not find a clear optimal temperature for clearance rate or the lack of a relation with temperature in any of the tested species. Our experiments confirmed earlier studies that clearance rates increases with temperature (Haure *et al.*, 1998; Ren *et al.*, 2000). This phenomenon has been explained by Riisgård and Larsen (2007) who showed that the decreasing viscosity of seawater with increasing temperature increases the beat frequency of water-pumping cilia of *M. edulis*. Our result shows that high temperature and low oxygen concentration combined reduced clearance rate in *M. edulis*. This is in line with the energy-saving mechanism proposed by Tang and Riisgård (2018).

We observed lower clearance rates at high food concentrations in the Dutch experiment. This is contrary to the expectation. For example, Joyce *et al.* (2019) showed an increase in clearance rate of both *M. edulis* and *C. gigas* with increasing food concentration. However, their food concentration was not expressed in chlorophyll concentration, rendering it difficult to compare results. High food concentrations can cause pseudo-faeces production and reduce clearance rates (Foster-Smith, 1975; Riisgård et al., 2011), but pseudofaeces production was not seen in our experiment and the concentration of the algae was set at 3750 cells per ml, which is well below the pseudofaeces threshold of 60.000 cells per ml determined for *M. edulis* juveniles by Blanco Garcia and Kamermans (2015).

The mussels standardized respiration rates at 15 °C, 100% saturation and high food were in the same range 0.71 ± 0.3 at 27 psu, as rates measured on mussels in Landes *et al.* (2015) at 12.5 °C, between 0.78 and 0.83 mg O_2/h/g at 25.7 and 29.5 psu. Surprisingly, oxygen consumption did not increase with temperature, except for NL mussels at low food concentration and low SO_2 at 25 °C. If the NL mussels were as described earlier showing some granulocytoma due to some stress, it could potentially be linked to an increase in respiration rate under sub-optimal conditions. Jeffrey *et al.* (2018) found an increase for freshwater mussels that were exposed to 31 °C for five days. Tremblay *et al.* (2011) exposed *M. edulis* to temperatures of either 12 °C or 25 °C for 31 days and also found higher standard metabolic rates at 25 °C than at 12 °C. Our experiment lasted 21-28 days, followed by a starvation

period of 25–40 days on the low food treatment, after which new RR measurements were taken (Fig. S1). Similar RR were measured and there was a significant increase of RR with temperature ($F_{2,173} = 16.73$, $p < 0.001$) and a significant decrease with decrease of SO_2 from H to L ($F_{2,\ 4.01} = 4.01$, $p = 0.03$). No significant interaction between temperature and low SO_2 was found. This suggests that food was an interacting factor in the experiment. Similarly, Tang and Riisgård (2018) found that mussels exposed to oxygen concentrations decreasing from 9 to 2 mg O_2/L resulted in a slow but significant reduction in the respiration rate at 13.5±0.6 °C.

The physiological response of the bivalve species to different temperatures, oxygen levels and food concentrations presented here can be used in dynamic energy budget models, such as presented by Maar *et al.* (2015) and Stechele *et al.* (submitted). Results of these individual models feed into farm scale models that project changes in productivity as a result of climate change (Cubillo *et al.*, 2021). The observation that different factor combinations yielded different responses is an important result to take into account in coastal areas where climate change is likely to affect those three factors, while it might be less critical in offshore areas (Palmer *et al.*, 2021). Cubillo *et al.* (2021) indicate that bivalve shellfish show a decreasing trend with respect to most productivity parameters as climate change progresses and offshore-suspended mussel culture in SW Portugal was least affected. Other factors such as invasive species, culture practice and diseases are likely to affect bivalve productivity on top of environmental effects with climate change. Modelling with an integrated approach (e.g. Ferreira *et al.*, 2021) including physiological response to multi environmental factors is of relevance for managers and the industry.

The Supplementary Material is available at https://www.alr.org/10.1051/alr/2022001/olm.

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