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RESEARCH ARTICLE

Future ocean warming may prove beneficial for the northern population of European seabass, but ocean acidification will not

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ABSTRACT

The world's oceans are acidifying and warming as a result of increasing atmospheric CO_2 concentrations. The thermal tolerance of fish greatly depends on the cardiovascular ability to supply the tissues with oxygen. The highly oxygen-dependent heart mitochondria thus might play a key role in shaping an organism's tolerance to temperature. The present study aimed to investigate the effects of acute and chronic warming on the respiratory capacity of European sea bass (*Dicentrarchus labrax* L.) heart mitochondria. We hypothesized that acute warming would impair mitochondrial respiratory capacity, but be compensated for by life-time conditioning. Increasing P_{CO_2} may additionally cause shifts in metabolic pathways by inhibiting several enzymes of the cellular energy metabolism. Among other shifts in metabolic pathways, acute warming of heart mitochondria of cold life-conditioned fish increased leak respiration rate, suggesting a lower aerobic capacity to synthesize ATP with acute warming. However, thermal conditioning increased mitochondrial functionality, e.g. higher respiratory control ratios in heart mitochondria of warm life-conditioned compared with cold life-conditioned fish. Exposure to high P_{CO_2} synergistically amplified the effects of acute and long-term warming, but did not result in changes by itself. This high ability to maintain mitochondrial function under ocean acidification can be explained by the fact that seabass are generally able to acclimate to a variety of environmental conditions. Improved mitochondrial energy metabolism after warm conditioning could be due to the origin of this species in the warm waters of the Mediterranean. Our results also indicate that seabass are not yet fully adapted to the colder temperatures in their northern distribution range and might benefit from warmer temperatures in these latitudes.

KEY WORDS: Mitochondrial respiration, Developmental acclimation, Temperate teleost, Heart

INTRODUCTION

The increasing amount of atmospheric CO_2 is working as a greenhouse gas, raising atmospheric temperatures and, as a consequence, also sea surface temperatures (ocean warming). At the same time, about a third of the atmospheric CO_2 is taken up by the oceans, which leads to decreasing seawater pH through the formation of carbonic acid (ocean acidification). These processes together will lead to warmer and more acidic oceans, a trend that has already been observed over the last decades and is predicted to continue. Depending on the representative concentration pathway, the IPCC (2014) predicts that the partial pressure of CO_2 (P_{CO_2}) will increase up to 1000 µatm above current values until the end of this century. Temperature projections for the same time span predict increases of up to 4°C at the coast of Brittany (Sheppard, 2004). Changes in water temperature have direct influence on the metabolic rate of ectothermic organisms, such as fish, with consequences for growth (Pörtner et al., 2007; Peck, 2002), reproductive success (for review, see Llopiz et al., 2014) and biogeography (Turner et al., 2009; Pörtner, 2006). The body of studies looking into the effects of the changing environment on marine ectothermic organisms is growing rapidly. However, only a small number of these studies have investigated the effects of ocean acidification and the combined effects of ocean acidification and warming on fish, with contrasting results between species as well as life stages (Kreiss et al., 2015; Heuer and Grosell, 2014; Pope et al., 2014; Bignami et al., 2013; Frommel et al., 2012). More investigation on a variety of fish species, on different life stages and under ecologically relevant P_{CO_2} concentrations appears necessary (Pope et al., 2014).

The effects of temperature on fish metabolism have been investigated intensively (e.g. Johnson and Katavic, 1986; Mirkovic and Rombough, 1998; Bláquez et al., 1998; Farrell, 2002; Pörtner et al., 2007; Hilton et al., 2010; Strobel et al., 2012). Thermal sensitivity of fish is mainly dependent on the capacity of the cardiovascular system to supply the tissues with oxygen (Pörtner and Lannig, 2009). The heart is a highly aerobic tissue (Driedzic, 1992) and it is therefore believed that the capacity of the heart mitochondria to produce ATP plays a central role in defining thermal tolerance in fish. Although the subcellular processes are not yet fully understood, it has been suggested that the functionality of heart mitochondria determines the temperature of heart failure, and that heart thermal acclimation capacity is relatively limited to safeguard functionality (e.g. Chen and Knowlton, 2010; Chung et al., 2017; Iftikar and Hickey, 2013; Strobel et al., 2013a). Different mitochondrial processes have been shown to be impaired at elevated temperatures, as indicated by decreased oxidative phosphorylation, decreased ATP production efficiency and lost integrity of the protein complexes of the electron transport system (e.g. Fangue et al., 2009; Hilton et al., 2010; Mark et al., 2012; Iftikar and Hickey, 2013). Additionally, high temperatures increase the fluidity of mitochondrial membranes, potentially leading to increased proton leak through the inner mitochondrial membrane and decreased mitochondrial efficiency (Pörtner, 2012). Impaired mitochondrial metabolism thus might lead to alterations in cardiomyocyte ATP supply and consequently affect the performance of the cardiovascular system, which will ultimately determine the thermal sensitivity of the fish. Although juvenile and adult fish generally possess well-developed acid–base regulating mechanisms (for review, see Heuer and Grosell, 2014), increased

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List of abbreviations

A	ambient P_{CO_2} condition
C	cold life-conditioned group
CCO	cytochrome *c* oxidase
CI–IV	complex I–IV of the electron transport system
CT_{max}	critical thermal maximum
dd	degree days
dph	days post-hatching
L_{Omy}	LEAK respiration with oligomycin
LME model	linear mixed effect model
P	OXPHOS respiration
RCP	representative concentration pathway
RCR_{Omy}	respiratory control ratio with oligomycin
W	warm life-conditioned group
Δ500	ambient P_{CO_2}+500 µatm acidification condition
Δ1000	ambient P_{CO_2}+1000 µatm acidification condition

ocean acidification may act as an additional stressor, e.g. by increasing the ATP demand for acid–base regulation. Consequently, well-functioning mitochondria are even more important if temperature and P_{CO_2} are increased simultaneously. However, while the body of literature on the effects of temperature on mitochondrial function in fish is relatively large (e.g. Fangue et al., 2009; Shama et al., 2014, and references therein), only a handful of studies have investigated the effects of increased P_{CO_2} on fish mitochondria and even fewer have combined ocean acidification and ocean warming (but see Strobel et al., 2013a; Leo et al., 2017). CO_2 can freely diffuse out of the water into the extracellular, intracellular and mitochondrial space. Just as in the blood, decreased intracellular pH due to elevated P_{CO_2} is buffered by actively raising intracellular bicarbonate levels (Strobel et al., 2013a). Mitochondrial membranes are permeable to CO_2 but impermeable to bicarbonate (Arias-Hidalgo et al., 2016), resulting in elevated matrix bicarbonate levels under high CO_2 (Pörtner and Sartoris, 1999; Strobel et al., 2012). Bicarbonate acts as a competitive inhibitor of mitochondrial citrate synthase and succinate dehydrogenase in rodents (Simpson, 1967; Wanders et al., 1983), which can be overcome by a compensatory increase in mitochondrial capacity following hypercapnia acclimation – although this has not yet been documented unequivocally for marine fish (Strobel et al., 2012; 2013a,b). Bicarbonate also acts as a pH-sensing molecule in marine fish and invertebrates (Tresguerres et al., 2014; Barott et al., 2017), with both upregulating and downregulating consequences on mitochondrial metabolism. As over the coming decades ocean temperature and P_{CO_2} will rise hand in hand, it is important to determine their combined effects on mitochondrial metabolism to be able to take ecologically relevant conclusions. Furthermore, there is a relative lack of studies that focus on temperate, large and/or economically relevant species, while at the same time employing realistic P_{CO_2} and exposure scenarios.

In our study, we exposed European seabass, *Dicentrarchus labrax* (L.), from 3 days post-hatching (dph) for 7 months to two temperatures and three P_{CO_2} conditions in a full-factorial design. Temperature and P_{CO_2} conditions reflect the predictions of the IPCC for 2100 (IPCC, 2014). The European seabass is an important aquaculture species (160,000 metric tons in 2015), but also an important target in commercial as well as in recreational fishing activities (Bjørndal and Guillen, 2018). European seabass are distributed throughout the Mediterranean, the Black Sea and the North-Eastern Atlantic from Norway to Senegal in coastal waters from coastal lagoons and estuaries up to 100 m depth (Bjørndal and Guillen, 2018). It has a relatively high tolerance to different temperatures and salinities (Dalla Via et al., 1998; Claireaux and Lagardère, 1999). We studied the effect of ocean acidification and warming on mitochondria of juvenile seabass by determining mitochondrial respiratory capacity in permeabilized heart fibers of seabass juveniles that had been reared under the respective ocean acidification and warming conditions since hatching (7 months). We examined the effects of ocean acidification and warming on mitochondrial ATP-producing processes (OXPHOS respiration) and the counteracting proton leak (LEAK respiration), as well as their resulting ratio (the respiratory control ratio, RCR). An increased LEAK respiration, which is not compensated for by increased OXPHOS respiration, results in a drop in RCR and indicates that mitochondrial functionality and consequently mitochondrial capacity to produce ATP are impaired.

We hypothesized that acute warming would impair mitochondrial performance in juvenile seabass hearts, as LEAK respiration may increase with thermal deterioration of mitochondrial membranes. However, compensational processes after long-term and developmental thermal conditioning could include changes in mitochondrial membrane properties, which would reduce LEAK respiration rates and consequently restore RCR. Additionally, we wanted to fathom the capacity of seabass mitochondria to cope with ocean acidification, especially when combined with ocean warming. We hypothesized that the changes in intracellular P_{CO_2} and bicarbonate concentration elicited by ocean acidification would affect mitochondrial metabolism, e.g. by inhibiting citrate synthase and succinate dehydrogenase, putting further pressure on the cellular energy metabolism.

MATERIALS AND METHODS

The present work was performed within Ifremer-Centre de Bretagne facilities (agreement number: B29-212-05). Experiments were conducted according to the ethics and guidelines of French law and legislated by the local ethics committee (Comité d'Ethique Finistérien en Experimentation Animal, CEFEA, registering code C2EA-74; authorization APAFIS 4341.03, permit number 2016120211505680.v3).

All chemicals were purchased from Sigma-Aldrich, except for tricaine methane sulfonate (MS-222), which was purchased from Pharma Q Limited.

Datasets of mitochondrial respiration and water conditions during rearing, as well as additional information on larval rearing are available online in PANGAEA (www.pangaea.de/).

Animals and experimental conditions

Animals

Larvae were obtained from the aquaculture facility Aquastream (Ploemeur-Lorient, France) at 2 dph (20 January 2016). Brood stock fish were caught in the sea off Morbihan, France. Four females (mean mass 4.5 kg) were crossed with 10 males (mean mass 2.4 kg), which spawned naturally using photothermal manipulation. Conditions in the aquaculture facility during breeding were as follows: 13°C, 35 psu, pH 7.6, 8 h 45 min of light followed by 15 h 15 min darkness. Spawning of eggs took place on 15 January 2016; larvae hatched on 18 January 2016 and were transported to our structures on 20 January 2016.

Larval rearing

Larval rearing was performed in a temperature-controlled room using black 35 l tanks initially stocked with ca. 5000 larvae per tank. Three replicate tanks were used for each temperature–P_{CO_2} combination. Larvae were randomly distributed into the

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experimental tanks at 3 dph (21 January 2016). During the following 3 days, the temperature for the warm life condition was increased stepwise, 1°C during the first day and 2°C during each of the two subsequent days. The P_{CO_2} conditions were applied directly after division into the experimental tanks. Starting at 7 dph (mouth opening), larvae were fed with live artemia, hatched from High HUFA Premium artemia cysts (Catvis, AE's-Hertogenbosch, The Netherlands). Artemia were fed to the larvae 24 h after rearing cysts in sea water up to 33 dph; afterwards, the artemia nauplii themselves were fed with cod liver oil and dry yeast after 24 h and fed to the larvae after 48 h. The artemia were transferred to the larval rearing tanks from two storage tanks (one for each temperature) with peristaltic pumps; their concentration in the tanks was maintained high during the day to allow *ad libitum* feeding; excess artemia left the tank via the waste water outflow. The 15 h photoperiod in the larval rearing room lasted from 07:00 h to 22:00 h; the light intensity increased progressively during the larval rearing period from total darkness to 96 lx (Table S1). Headlamps were used (set to the lowest light intensity) to allow us to work in the larval rearing facility. Larval mortality was 10–80%, regardless of the rearing condition (Table S2). The water surface was kept free of oily films using a protein skimmer. Larval density was reduced regularly during larval rearing as samples for other experiments were taken throughout the entire larval rearing period (at approximately 100, 300, 500, 700, 750 and 900 degree days, dd=dph×temperature in °C). At approximately 980 dd, the early juveniles were transferred from the larval to the juvenile rearing facility, corresponding to 50 dph and 65 dph for 20°C and 15°C rearing, respectively.

Juvenile rearing

As they reached juvenile stage, fish were moved from the larval rearing facilities to juvenile tanks at approximately 1000 dd (50 dph, 8 March 2016, and 65 dph, 23 March 2016, for warm and cold life conditions, respectively). Fish were counted per tank and all fish from one condition were pooled in one tank until the swim bladder test and separation into duplicate tanks at 1541 dd (78 dph, 5 April 2016) and 1301 dd (86 dph, 13 April 2016) for warm and cold life conditions, respectively. The swim bladder test was done to keep only the fish with developed swim bladders. Briefly, the fish were anesthetized and introduced into a test container with seawater with a salinity of 65 psu. Those fish floating at the surface were removed from the test container and placed into the rearing tanks for recovery. The juveniles were reared in round tanks with a volume of 0.67 m^3 and a depth of 0.65 m. Mortality rates of 24.8–43.4% (Table S3) occurred between moving to the juvenile facility and the swim bladder test. During the first 5 days after moving to juvenile rearing facilities, the juveniles were fed artemia nauplii (48 h old and enhanced with cod liver oil and dry yeast) and commercial fish food. Commercial fish food (Neo Start) was fed daily and was adjusted in size (1–3) and amount during the juvenile rearing time, as recommended by the supplier (Le Gouessant, Lamballe, France). More precisely, fish were fed *ad libitum* until 19 August 2016; afterwards, food ratios were calculated every 3–4 weeks for each tank in respect to biomass and temperature of the tank using the formulae provided by Le Gouessant. The daily ration of food was supplied to the tanks by automatic feeders during the daytime; the fish were not fed during the night-time. Photoperiod was adjusted to natural conditions once a week, with slowly increasing light intensity in the juvenile rearing facilities during the first hour each morning. The tanks were cleaned daily after pH measurements. Water flow within the tanks was adjusted once a week, so that oxygen saturation levels were not below 90%, keeping equal flow-through rates in all tanks of one temperature.

Experimental conditions

The larvae and juveniles were reared under six different ocean acidification and warming scenarios, following the predictions of the IPCC (2014) for the next 130 years. The acidification conditions included three different P_{CO_2}: today's ambient situation in coastal waters of Brittany and the Bay of Brest (ambient group – A, approximately 650 µatm; see Pope et al., 2014; Duteil et al., 2016), a scenario according to the IPCC representative concentration pathway RCP 6.0, projecting a ΔP_{CO_2} of 500 µatm to current values (Δ500, approximately 1150 µatm) and a scenario according to RCP8.5, projecting a ΔP_{CO_2} of 1000 µatm (Δ1000, approximately 1700 µatm). All acidification conditions were crossed with two different temperatures to create a 'cold' (C) and a 'warm' (W) life condition scenario. In the cold life condition, larvae were reared at 15°C; juveniles in the cold life condition were reared at 15°C until ambient temperature in the Bay of Brest reached 15°C, and from there on juveniles were reared at ambient temperatures of the Bay of Brest (up to 18°C in 2016). The warm life condition mirrored these thermal profiles, but with an offset of plus 5°C (20–23°C). As larvae and post-larval juveniles would display different growth rates under the two different thermal scenarios, we adopted the concept of degree days (dd, see above) as the basis for comparison between these life conditions.

The sea water used in the aquaria was pumped in from the Bay of Brest from a depth of 20 m approximately 500 m from the coastline, passed through a sand filter (~500 µm), heated (tungsten, Plate Heat Exchanger, Vicarb, Sweden), degassed using a column, filtered using a 2 µm membrane and finally UV sterilized (PZ50, 75 W, Ocene, Louvigné-du-Désert, France), ensuring high water quality. During larval and early juvenile rearing, the water supply for the acidified incubation tanks came from a central header tank, where the water P_{CO_2} conditions were adjusted. The water pH was controlled by an IKS Aquastar system (iks Computer Systeme GmbH, Karlsbad, Germany), which continuously measured pH in one of the replicate tanks and opened a magnetic valve to bubble CO_2 into the header tank when pH in the rearing tank became too high. Water exchange was set to 20 l h^{-1} until 12 dph and 25 l h^{-1} until the end of larval rearing. During juvenile rearing with higher water exchange rates, additional PVC columns were installed to control the pH in the rearing tanks. The water arrived at the top of the column and was pumped from the bottom of the column to the rearing tanks. The CO_2-bubbling apparatus was installed at the bottom of the column and was adjusted by a flow control unit when pH deviated from the desired value. One column supplied both replicate tanks of each condition. Temperature and pH were checked each morning with a handheld WTW 3110 pH meter (with a WTW Sentix 41 electrode, NIST scale; both from Xylem Analytics Germany, Weilheim, Germany) before fish were fed. The pH meter as well as the IKS Aquastar system were calibrated daily with NIST-certified WTW technical buffers pH 4.01 and pH 7.00 (Xylem Analytics Germany). Total alkalinity was measured once a week following the protocol of Anderson and Robinson (1946) and Strickland and Parsons (1972): 50 ml of filtered tank water (200 µm nylon mesh) was mixed with 15 ml HCl (0.01 mol l^{-1}) and pH was measured immediately. Total alkalinity was then calculated with the following formula:

$$\mathrm{TA} = \frac{V_{\mathrm{HCl}} \cdot c_{\mathrm{HCl}}}{V_{\mathrm{sample}}} - \frac{(V_{\mathrm{HCl}} + V_{\mathrm{sample}})}{V_{\mathrm{sample}}} \cdot \frac{[\mathrm{H}^+]}{\gamma^{\mathrm{H}^+}}, \quad (1)$$

where TA is total alkalinity (mol l^{-1}), V_{HCl} is the volume of HCl (l), c_{HCl} is the concentration of HCl (mol l^{-1}), V_{sample} is the volume of the sample (l), [H^+] is hydrogen activity (10^{-pH}) and γ^{H^+} is the hydrogen activity coefficient (here $\gamma^{H^+}=0.758$).

The Microsoft Excel macro CO2sys (Lewis and Wallace, 1998) was used to calculate seawater carbonate chemistry, using the constants proposed by Mehrbach and colleagues, refitted by Dickson and Millero (see CO2sys). Oxygen saturation (WTW Oxi 340, Xylem Analytics Germany) and salinity (WTW LF325, Xylem Analytics Germany) were measured once a week together with total alkalinity, from juvenile stage onwards (see Table 1 for all water parameters).

Mitochondrial respirometry

Measurements of mitochondrial respiration rates were performed from approximately 3700 to 4100 dd in all conditions (183–199 dph and 234–249 dph in warm and cold life conditions, respectively). Although the same age in degree days was chosen, the cold life-conditioned fish were significantly smaller than the warm life-conditioned fish [length: 8.72±0.09 cm and 9.59±0.09 mm, respectively, least squares (ls)means±s.e.m., $P<0.001$; and carcass mass: 10.00±0.45 g and 13.38±0.46 g, respectively, lsmeans±s.e.m., $P<0.001$, linear mixed effect models (LME)], resulting in smaller ventricle sizes (0.0105±0.0004 g and 0.0122±0.005 g, respectively, $P<0.05$, lsmeans±s.e.m., LME), as well as lower hepatosomatic indices (1.51±0.05 and 2.45±0.06, respectively, lsmeans±s.e.m., $P<0.0001$, LME). However, condition factor was not different in the two temperature conditions (1.51±0.01 and 1.48±0.01, respectively, lsmeans±s.e.m., $P>0.1$, LME) (see Table S4). P_{CO_2} did not have an effect on fish size. Prior to the experiments, the fish were starved for 2 days. Two batches of eight fish each were processed per day. Juveniles were randomly caught from their tanks and anesthetized with MS-222. The concentration of the anesthetic was adjusted to reach a loss of equilibrium within less than 5 min, typically 0.2 g l^{-1}. Mass, fork length and body length were directly determined with a precision balance (Mettler, Columbus, OH, USA) and a caliper, to the nearest 0.01 g and 0.01 mm, respectively. Afterwards, fish were killed by a cut through the neck, and the heart was completely dissected from the fish, followed by excavation of the ventricle. Excess blood was removed from the ventricle by cleaning it on blotting paper, prior to weighing (Sartorius, Göttingen, Germany). Tissue from a whole ventricle was used for respiration measurements in each respiration chamber of the oxygraphs and respiration rates were normalized to ventricle mass. The ventricle was stored in relaxing and biopsy preservation solution [BIOPS: 10 mmol l^{-1} Ca-EGTA (0.1 µmol l^{-1} free calcium), 20 mmol l^{-1} imidazole, 20 mmol l^{-1} taurine, 50 mmol l^{-1} K-Mes, 0.5 mmol l^{-1} DTT, 6.56 mmol l^{-1} $MgCl_2$, 5.77 mmol l^{-1} ATP, 15 mmol l^{-1} phosphocreatine, pH 7.1; modified after Gnaiger et al., 2000] until all eight ventricles were dissected. The ventricles were manually frayed and were permeabilized on ice with saponin (50 µg ml^{-1}) for 20 min on a shaking table (80 rpm), followed by two cleaning steps for 10 min at 80 rpm in modified mitochondrial respiration medium [MiR05: 160 mmol l^{-1} sucrose, 60 mmol l^{-1} potassium-lactobionate, 20 mmol l^{-1} taurine, 20 mmol l^{-1} Hepes, 10 mmol l^{-1} KH_2PO_4, 3 mmol l^{-1} $MgCl_2$, 0.5 mmol l^{-1} EGTA, 1 g l^{-1} bovine albumin serum (fatty acid free), pH 7.45 at 15°C; modified after Fasching et al., 2014]. During the permeabilization step, the livers and the carcasses of the fish were weighed to calculate the hepatosomatic index (HSI) and condition factor (K) (see Table S4).

Mitochondrial respiration of the permeabilized heart fibers was measured using four Oroboros Oxygraph-2K respirometers with DatLab 6 software (Oroboros Instruments, Innsbruck, Austria). Permeabilized fibers have the advantage of resembling the living state as closely as possible, while still allowing control of the supply of substrates and inhibitors to the mitochondria (Saks et al., 1998; Pesta and Gnaiger, 2012). Measurements were conducted at 15 and 20°C for all treatments to determine the effect of acute temperature changes on mitochondrial metabolism *in vitro*. The oxygen sensors were calibrated in air-saturated buffer prior to the experiments and in oxygen-depleted buffer after each experiment. The measurements were done in MiR06 buffer (MiR05 buffer enriched with 280 U ml^{-1} catalase; Fasching et al., 2014) to allow for reoxygenation with H_2O_2. A standard substrate–uncoupler–inhibitor titration protocol was employed to measure the respiration rates of the different complexes: glutamate (10 mmol l^{-1}), malate (2 mmol l^{-1}) and pyruvate (10 mmol l^{-1}) were used to measure LEAK respiration of complex I [$L(n)_{CI}$; Table 2], followed by addition of ADP

Table 1. Water parameters during larval and juvenile phase of the 2016 batch

Treatment	pH	Temp. (°C)	Salinity (psu)	O_2 (% air sat.)	TA (mol l^{-1})	P_{CO_2} (µatm)
Larvae						
C–A	7.95±0.01	15.3±0.0	33.0±0.1	–	2364±17	656±16
C–Δ500	7.77±0.01	15.3±0.0	33.0±0.1	–	2382±19	1041±26
C–Δ1000	7.58±0.00	15.3±0.0	33.0±0.1	–	2394±26	1682±26
W–A	7.88±0.01	20.0±0.1	33.1±0.1	–	2369±21	832±13
W–Δ500	7.79±0.01	20.0±0.1	33.1±0.1	–	2383±22	1057±30
W–Δ1000	7.60±0.01	20.0±0.1	33.1±0.1	–	2380±23	1672±33
Juveniles						
C–A	7.97±0.01	16.0±0.2	34.2±0.1	90.9±0.5	2396±18	655±18
C–Δ500	7.75±0.01	16.0±0.2	34.2±0.1	92.2±0.6	2404±19	1107±21
C–Δ1000	7.55±0.01	16.1±0.2	34.2±0.1	90.9±0.6	2399±19	1841±40
W–A	7.92±0.01	21.9±0.2	35.0±0.2	90.2±0.9	2418±12	788±22
W–Δ500	7.78±0.01	21.8±0.2	35.0±0.2	90.5±0.7	2420±15	1133±43
W–Δ1000	7.59±0.01	21.9±0.2	35.0±0.2	91.3±0.6	2423±12	1808±65
Ambient seawater						
SW cold	8.05±0.01	14.5±0.5	33.0±0.2	101.2±0.6	2434±21	522±18
SW warm	7.95±0.02	21.2±0.4	32.7±0.1	102.3±1.4	2433±28	723±33

The larval period lasted until 4 March 2016 [46 days post-hatching (dph), ~900 degree days (dd)] and 18 March 2016 (60 dph, ~900 dd) for warm and cold life conditions, respectively; and the juvenile period until 24 October 2016 (280 dph, ~5900 dd) and 8 February 2017 (387 dph, ~6200 dd) for warm and cold life-conditioned fish, respectively. Data are means±s.e.m. over all replicate tanks per condition. pH, temperature (Temp.), salinity, oxygen saturation (during juvenile rearing) and total alkalinity (TA) were measured weekly; P_{CO_2} was calculated with CO2sys. Sea water (SW) measurements were conducted in 2017 and 2018. A, ambient P_{CO_2}; Δ500, ambient+500 µatm CO_2; Δ1000, ambient+1000 µatm CO_2; C, cold life condition; W, warm life condition.

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Table 2. Analyzed mitochondrial metabolic states (after Gnaiger, 2014) during the substrate–uncoupler–inhibitor titration protocol

Description	State	Name
Substrates for CI are available but no ADP	LEAK	$L(n)_{CI}$
Substrates for CI and ADP are available to fuel coupled respiration of CI	OXPHOS	P_{CI}
Substrates for CI and CII as well as saturating ADP are available to fuel coupled respiration of the full electron transport system	OXPHOS	P
Oligomycin inhibits F_1F_O-ATPase resulting in LEAK respiration	LEAK	L_{Omy}
Coupled respiration of CII calculated: $P-P_{CI}$	OXPHOS	P_{CII}
Respiratory control ratio calculated: $P\times L_{Omy}^{-1}$	–	RCR_{Omy}

Oxygen was available in all these states. $L(n)_{CI}$, LEAK respiration of complex I; P, OXPHOS respiration; CI, mitochondrial complex I; CII, mitochondrial complex II; L_{Omy}, LEAK respiration with oligomycin.

(2.5 mmol l^{-1}) to measure OXPHOS respiration of complex I (P_{CI}). Succinate (10 mmol l^{-1}) and further ADP (two additions of 2.5 mmol l^{-1}) resulted in OXPHOS respiration of complex I (CI) and complex II (CII) combined (P). Cytochrome c (0.01 mmol l^{-1}) was used as a control for inner mitochondrial membrane integrity; measurements with increases of more than 10% following cytochrome c addition were not used for further analyses. Oligomycin (4 µg ml^{-1}) was used to inhibit F_OF_1-ATPase, resulting in LEAK respiration (L_{Omy}). Stepwise titration of FCCP (0.05 µl of 2 mmol l^{-1} stock solution per step) was used to uncouple the mitochondrial electron transport system and determine its maximum capacity. After uncoupling, CI, CII and complex III (CIII) were successively inhibited with rotenone (0.005 mmol l^{-1}), malonate (5 mmol l^{-1}) and antimycin A (0.0025 mmol l^{-1}), respectively. Residual respiration after antimycin A addition was used to correct all mitochondrial respiration rates. Complex IV (CIV) capacity was then determined by addition of ascorbate (2 mmol l^{-1}) and TMPD (0.5 mmol l^{-1}). Oxygen levels were usually restored by addition of 2 µl H_2O_2 (3% stock solution) after the oligomycin and rotenone steps.

A measure for OXPHOS respiration of CII (P_{CII}) was calculated ($P_{CII}=P-P_{CI}$), although this measure will lead to lower respiration rates than direct determination of P_{CII} respiration, when only substrates for CII are available (Gnaiger, 2009; Mark et al., 2012). For all complexes, the contribution of the respiration of the respective complex to OXPHOS respiration was calculated. This was also done for L_{Omy}, which we tentatively termed the 'L_{Omy} fraction', as a relative indicator of proton leak, despite the fact that membrane potential is potentially higher in L_{Omy} than in natural state 4. Mitochondrial quality and efficiency were evaluated by calculating the respiratory control ratio ($RCR_{Omy}=P\times L_{Omy}^{-1}$), which is an indicator of mitochondrial coupling (Gnaiger, 2009; Strobel et al., 2013a).

Statistical analysis

All statistics were performed with R (http://www.R-project.org/). Data were tested for outliers (Nalimov test), normality (Shapiro–Wilk's test, $P>0.05$) and homogeneity (Levene's test, $P>0.05$). Mitochondrial respiratory data were fitted to linear mixed effect models (LME model, 'lme' function of 'nlme' package; https://CRAN.R-project.org/package=nlme). Conditioning and assay temperature, as well as P_{CO_2} and their interactions were included as fixed effects. Because of the significantly different sizes of the fish, fish mass was also included as a fixed effect, whereas the oxygraph chamber and the number of the run on that day were included as random effects. In case of heterogeneity of data, variance structures were included in the random part of the model; the best variance structure was chosen according to the lowest Akaike information criterion (AIC) values. Validity of linearity for P_{CO_2} concentration and mass was cross-tested with generalized additive models ('gam' function of 'mgcv' package; Wood, 2017), as described in Zuur et al. (2009). If linearity was given, the LME model was chosen instead of the generalized additive model. If significant effects were detected in the LME models, *post hoc* Tukey tests were performed with the 'lsmeans' function ('lsmeans' package; Lenth, 2016). Significance for all statistical tests was set at $P<0.05$. All graphs are produced from the lsmeans data with the 'ggplot2' package (Wickham, 2016). All data are shown as lsmeans±s.e.m. Biometrical data were also tested with LME models, with P_{CO_2}, conditioning and assay temperature as fixed effects and origin tank as a random effect. Model validation was carried out in the same way as described above for mitochondrial respiratory data.

RESULTS

Effects of acute *in vitro* warming on mitochondrial function in cold life-conditioned fish

P_{CII} (Fig. 3) respiration rates increased significantly with acute warming in the Δ1000 group (LME, $P<0.05$; Table 3). All other analyzed parameters were not affected by acute warming: P (Fig. 1), P_{CI} (Fig. 2) and the relative contributions of CI and CII to P (Table 3; CI fraction: 53.4±3.3–61.1±3.3%, Fig. S1; and CII fraction: 38.8±3.3–46.5±3.3%, Fig. S2).

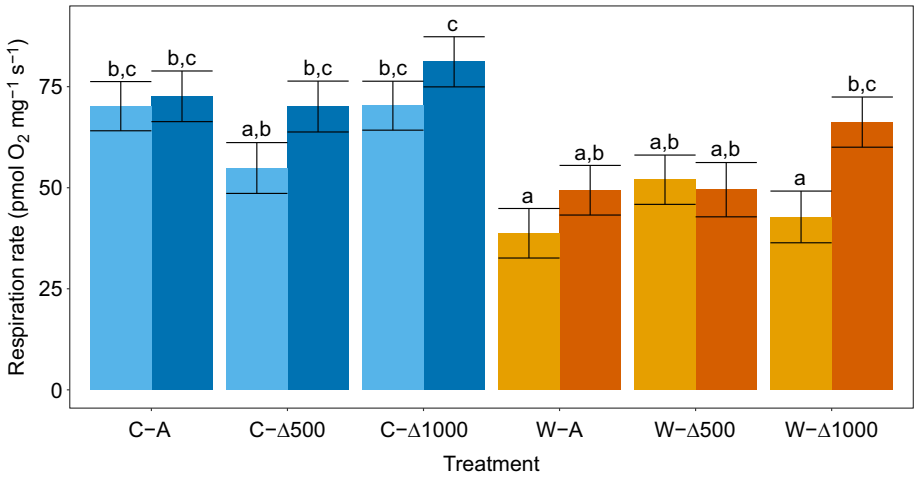

Fig. 1. Respiration rates of permeabilized heart ventricle of European seabass. Respiration rate (oxidative phosphorylation capacity, P) data are least squares (ls)means±s.e.m. Different letters indicate significant differences [linear mixed effects (LME), $P<0.05$]; blue: cold life-conditioned fish (C), orange: warm life-conditioned fish (W), light shading: cold assay temperature, dark shading: warm assay temperature. A, ambient P_{CO_2}; Δ500, ambient+500 µatm CO_2; Δ1000, ambient+1000 µatm CO_2. n_{C-A}=16/15, $n_{C-\Delta500}$=15/16, $n_{C-\Delta1000}$=16/16, n_{W-A}=16/15, $n_{W-\Delta500}$=17/11, $n_{W-\Delta1000}$=13/15, for cold/warm assay temperature, respectively.

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Table 3. *F*-values of fixed effects from the linear mixed models on mitochondrial respiration of permeabilized heart fibers of juvenile European seabass

	Den. d.f.	CT (1 d.f.)	AT (1 d.f.)	P_{CO_2} (2 d.f.)	Mass (1 d.f.)	CT:AT (1 d.f.)	CT:P_{CO_2} (2 d.f.)	AT:P_{CO_2} (2 d.f.)	CT:AT:P_{CO_2} (2 d.f.)
RCR_{Omy}	126	12.50^{***}	$1.66^{n.s.}$	$0.19^{n.s.}$	4.73^{*}	$1.31^{n.s.}$	$2.18^{n.s.}$	$0.05^{n.s.}$	$0.72^{n.s.}$
Oxygen consumption rate									
P	147	40.83^{***}	20.76^{***}	$2.46^{‡}$	$0.65^{n.s.}$	$0.09^{n.s.}$	$2.16^{n.s.}$	$2.04^{n.s.}$	4.20^{*}
P_{CI}	146	29.08^{***}	10.10^{**}	$1.17^{n.s.}$	$1.09^{n.s.}$	$0.00^{n.s.}$	3.33^{*}	$0.20^{n.s.}$	3.46^{*}
P_{CII}	144	12.30^{***}	26.30^{***}	$2.05^{n.s.}$	$0.22^{n.s.}$	$0.00^{n.s.}$	$0.15^{n.s.}$	4.14^{*}	3.52^{*}
L_{Omy}	141	122.51^{***}	39.75^{***}	$1.78^{n.s.}$	$2.87^{‡}$	5.43^{*}	4.03^{*}	$0.93^{n.s.}$	$0.18^{n.s.}$
CIV	129	38.64^{***}	42.90^{***}	$0.47^{n.s.}$	10.83^{**}	$2.82^{‡}$	$0.57^{n.s.}$	$0.08^{n.s.}$	$0.15^{n.s.}$
OXPHOS respiration fraction									
P_{CI} fraction	144	$2.93^{‡}$	$0.56^{n.s.}$	$0.14^{n.s.}$	$2.91^{‡}$	$0.00^{n.s.}$	$2.49^{‡}$	$2.23^{n.s.}$	$0.46^{n.s.}$
P_{CII} fraction	142	$2.37^{n.s.}$	$1.36^{n.s.}$	$0.07^{n.s.}$	$2.41^{n.s.}$	$0.02^{n.s.}$	3.16^{*}	$1.90^{n.s.}$	$0.45^{n.s.}$
L_{Omy} fraction	144	9.82^{**}	4.59^{*}	$0.65^{n.s.}$	$1.76^{n.s.}$	$3.28^{‡}$	$2.57^{‡}$	$0.70^{n.s.}$	$1.27^{n.s.}$
CIV capacity	130	$2.85^{‡}$	$1.48^{n.s.}$	$1.00^{n.s.}$	$2.75^{‡}$	$0.19^{n.s.}$	$1.80^{n.s.}$	$1.11^{n.s.}$	$1.14^{n.s.}$

Denominator degrees of freedom (Den. d.f.) and the number of degrees of freedom (1 or 2) are indicated. CT, conditioning temperature; AT, assay temperature; RCR_{Omy}, respiratory control ratio with oligomycin; P, oxidative phosphorylation capacity; CIV, complex IV; L_{Omy}, LEAK respiration with oligomycin. $^{‡}P<0.1$; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

CIV capacity increased with acute warming in both hypercapnia groups (LME, Δ500: $P<0.05$ and Δ1000: $P<0.05$; Table 3) but not in fish at ambient P_{CO_2} (Fig. 4). However, this increase was not strong enough to change the relationship between CIV capacity and P (Fig. S3).

L_{Omy} respiration rate increased significantly with acute warming in the A and Δ1000 treatments (LME, $P<0.05$ and $P<0.01$, respectively; Table 3 and Fig. 5). Assay temperature had a significant effect on the relative contribution of L_{Omy} to P (L_{Omy} fraction; Table 3). However, no specific differences were detected with the *post hoc* tests (Fig. S4). As P_{CO_2} did not affect the L_{Omy} fraction, we pooled data over P_{CO_2} treatments, which emphasized that acute warming led to impaired mitochondria in the cold life-conditioned fish, as indicated by a significantly higher L_{Omy} fraction under warm assay temperatures compared with cold assay temperatures (Table 4; LME, $P<0.05$).

Effects of acute *in vitro* cooling on mitochondrial function in warm life-conditioned fish

Acute cooling led to significantly decreased P and P_{CII} respiration rates in the Δ1000 group (LME, $P<0.01$, Fig. 1 and $P<0.01$, Fig. 3, respectively). All other analyzed parameters were not affected by acute cooling: P_{CI} (Fig. 2), CIV (Fig. 4), L_{Omy} (Fig. 5), and the relative contributions of CI, CII, CIV and L_{Omy} to P (Table 3; CI fraction: 50.2±3.2–57.5±3.3%, Fig. S1; CII fraction: 41.7±3.2–49.7±3.1%; Figs S2, S3 and S4).

Effects of long-term thermal conditioning on mitochondrial function

Warm life conditioning led to decreased P and P_{CI} respiration rates in the A and Δ1000 group, either at both assay temperatures (P_{CI} in the A group, Fig. 2, LME, $P<0.01$) or only at the cold assay temperature (P in the A and Δ1000 group, Fig. 1, LME, $P<0.001$ and $P<0.01$, respectively; and P_{CI} in the Δ1000 group, LME, cold assay temperature, $P<0.05$).

No significant effects of warm life conditioning were observed for P_{CII} (Fig. 3) and CIV capacity (Fig. 4) or the relative contributions of CI and CII to P respiration (Table 3). CIV capacity was 1.5–2 times higher than P respiration (Fig. S3) and did not differ between thermal life conditions.

L_{Omy} respiration rate was significantly decreased in warm life-conditioned fish compared with cold life-conditioned fish in the A group (Fig. 5; LME, $P<0.01$ and $P<0.0001$, both assay temperatures, respectively) and in the Δ500 group (LME, $P<0.0001$, warm assay temperature).

Thermal life condition and assay temperature had significant effects on the relative contribution of L_{Omy} to P (L_{Omy} fraction; Table 3). As P_{CO_2} did not affect the L_{Omy} fraction, P_{CO_2} treatment data

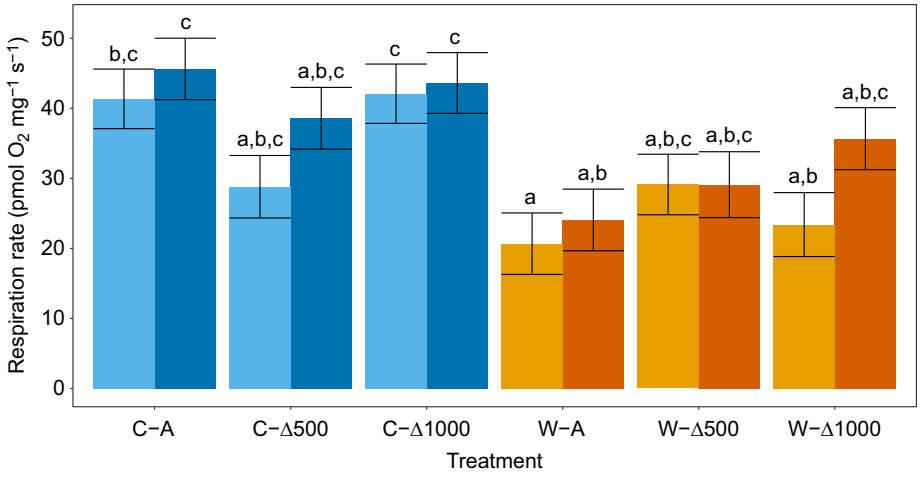

Fig. 2. OXPHOS respiration rate of complex I (P_{CI}) in permeabilized heart ventricle fibers of European seabass. Data are lsmeans±s.e.m. Different letters indicate significant differences (LME, $P<0.05$); blue: cold life-conditioned fish (C), orange: warm life-conditioned fish (W), light shading: cold assay temperature, dark shading: warm assay temperature. A, ambient P_{CO_2}; Δ500, ambient+500 µatm CO_2; Δ1000, ambient+1000 µatm CO_2. $n_{C-A}=16/15$, $n_{C-\Delta500}=14/16$, $n_{C-\Delta1000}=16/16$, $n_{W-A}=16/14$, $n_{W-\Delta500}=17/11$, $n_{W-\Delta1000}=13/15$, for cold/warm assay temperature, respectively.

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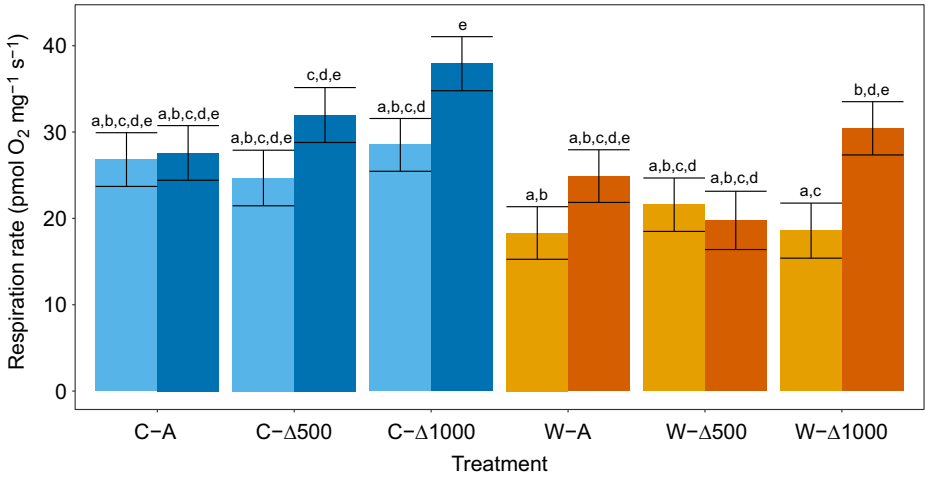

Fig. 3. OXPHOS respiration rate of complex II (P_{CII}) in permeabilized heart ventricle fibers of European seabass. Data are lsmeans±s.e.m. Different letters indicate significant differences (LME, P<0.05); blue: cold life-conditioned fish (C), orange: warm life-conditioned fish (W), light shading: cold assay temperature, dark shading: warm assay temperature. A, ambient P_{CO_2}, Δ500: ambient+500 µatm CO_2, Δ1000: ambient+1000 µatm CO_2. $n_{C–A}$=15/15, $n_{C–Δ500}$=14/16, $n_{C–Δ1000}$=16/16, $n_{W–A}$=15/14, $n_{W–Δ500}$=16/11, $n_{W–Δ1000}$=13/15, for cold/warm assay temperature, respectively.

were pooled. These data indicated by a significantly higher L_{Omy} fraction in the warm compared with the cold assay temperature that acute warming led to impaired mitochondria in the cold life-conditioned fish, whereas the L_{Omy} fraction did not differ between the thermal life history groups when compared at the respective conditioning temperature (Table 4; LME, P<0.05). Conditioning to warmer temperatures led to a significantly decreased L_{Omy} fraction (Table 3).

Mitochondria were well coupled in all treatments (RCR_{Omy}>4), with significantly higher RCR_{Omy} in warm life-conditioned fish than in cold life-conditioned fish (LME, 9.43±1.38 and 6.46±1.33, respectively, P<0.05). There were no significant effects of assay temperature, P_{CO_2} or any interaction terms on RCR_{Omy} (Table 3).

Effects of acclimation to different P_{CO_2} on mitochondrial function

Elevated P_{CO_2} alone did not have significant effects on any of the studied complexes and processes of the electron transport chain (Table 3). However, we found synergistic effects with temperature which became visible as interaction effects with life condition or assay temperature only in the Δ500 or Δ1000 fish, as specified above.

DISCUSSION

Mitochondrial functional capacities were examined in seabass juveniles raised in six combinations of three P_{CO_2} and two temperature treatments. The data provide evidence that heart mitochondria of juvenile seabass can be impaired by acute warming, as observed in increased L_{Omy} respiration rates, for example. In contrast, warm life conditioning increased mitochondrial efficiency in comparison to that of cold life-conditioned fish, as seen through increased RCR_{Omy}.

Ocean acidification did not affect mitochondrial functioning in juvenile seabass as a single factor, as indicated by no significant effects of P_{CO_2} alone on mitochondrial capacities. However, ocean acidification intensified the effects of acute or long-term warming. This was most prominent in the high acidification warm life condition treatment, e.g. P respiration rates were only significantly affected by acute temperature change in the W–Δ1000 fish, due to the decrease of P_{CII} in this group. CI, CIV and RCR_{Omy} were not affected by P_{CO_2}. This observation reflects the findings of previous studies in polar fish, where thermal effects on mitochondrial capacity were much more prominent than those of ocean acidification (e.g. Leo et al., 2017; Strobel et al., 2013b). However, the reduced ability of CII to cope with acute temperature changes in the W–Δ1000 fish is in agreement with other studies which found that CII was inhibited by elevated P_{CO_2} in mammals and fish (Simpson, 1967; Wanders et al., 1983; Strobel et al., 2013a). In juvenile seabass, CII was only inhibited by high P_{CO_2} in warm life-conditioned fish facing an acute temperature decrease. Therefore, juvenile seabass mitochondria appear generally able to cope with the inhibiting effect of high P_{CO_2} on CII. Other studies suggested that mitochondria could employ anaplerotic

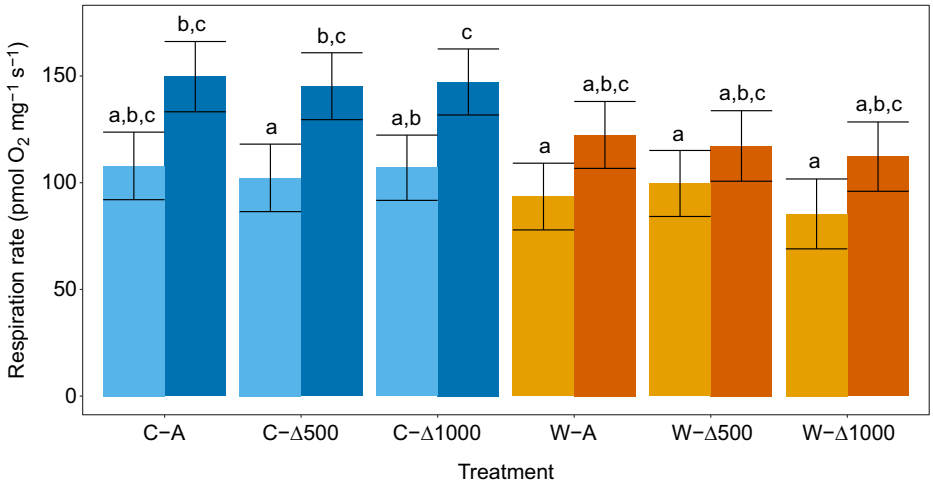

Fig. 4. Complex IV (CIV) respiration rates of permeabilized heart ventricle fibers of European seabass. Data are lsmeans±s.e.m. Different letters indicate significant differences (LME, P<0.05); blue: cold life-conditioned fish (C), orange: warm life-conditioned fish (W), light shading: cold assay temperature, dark shading: warm assay temperature. A, ambient P_{CO_2}; Δ500: ambient+500 µatm CO_2; Δ1000: ambient+1000 µatm CO_2. $n_{C–A}$=12/12, $n_{C–Δ500}$=14/16, $n_{C–Δ1000}$=15/16, $n_{W–A}$=15/14, $n_{W–Δ500}$=17/11, $n_{W–Δ1000}$=11/13, for cold/warm assay temperature, respectively.

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Table 4. L_{Omy} fraction of *P* respiration in permeabilized heart fibers of European seabass

AT	C (%)	W (%)
Cold	16.58±1.60[a]	14.58±1.65[a]
Warm	20.79±1.65[b]	14.41±1.70[a]

Data are least squares (ls)means±s.e.m. pooled over all P_{CO_2} conditions; different letters indicate significant differences (linear mixed effects, LME, $P<0.05$). C, cold life-conditioned fish; W, warm life-conditioned fish; AT, assay temperature. $n_{C–Cold}=46$, $n_{C–Warm}=45$, $n_{W–Cold}=46$, $n_{W–Warm}=40$.

mechanisms, such as decarboxylation of aspartate and glutamate, feeding into the Krebs cycle via oxaloacetate and oxoglutarate and stimulating CI with additional NADH-related substrates to overcome inhibitory effects of high P_{CO_2} of CII (Langenbuch and Pörtner, 2002; Strobel et al., 2013a). However, if environmental temperature or P_{CO_2} concentration further increase, decreased *P* respiration rates due to reduced CII respiration could occur at the respective habitat (conditioning) temperature and not only under acute temperature change.

Acute warming impairs heart mitochondria of cold life-conditioned juvenile seabass by increasing L_{Omy} respiration, significantly so in the C–A and C–Δ1000 groups, as seen in the increased L_{Omy} fraction. Increased leak respiration rates (L_{Omy} as well as L_{Omy} fraction) indicate decreasing mitochondrial membrane integrity, translating into less ATP produced for the same amount of oxygen consumed. Increases in mitochondrial enzyme activity and respiration rates of mitochondrial complexes, as well as *P* respiration rates with acute temperature increase have been shown in other fish species, e.g. Antarctic nototheniids, European perch and Atlantic cod (Strobel et al., 2013a; Ekström et al., 2017; Leo et al., 2017). Iftikar and Hickey (2013) showed in hearts of New Zealand triple fin fishes that compromised mitochondria at acutely elevated temperature will ultimately lead to heart failure. Consequently, as acute warming of only 5°C impaired mitochondria of the cold life-conditioned fish, it appears likely that cold life-conditioned juvenile seabass can suffer from heart metabolic deficiencies, if acute temperature changes exceed 5°C. This reduced tolerance to acute temperature increase in the cold life-conditioned fish seems to contradict the fact that European seabass are generally highly tolerant to a wide range of temperatures (Dalla Via et al., 1998; Claireaux and Lagardère, 1999). It also seems to contradict the high critical thermal maximum (CT_{max}) of European seabass (28.12±0.09 to 32.50±0.04°C in Mauduit et al., 2016; and 31.3±0.3°C in Anttila et al., 2017). However, in seabass acclimated to 17°C, Anttila et al. (2017) found arrhythmia occurred at around 22°C, although CT_{max} was above 30°C. Furthermore, the Arrhenius breakpoint temperature for maximum heart rate, which is connected to the thermal optimum of growth and aerobic scope, was 19.3±0.3°C and the temperature with the highest maximal heart rate, a measure of the thermal limits of cardiac function, was 21.8±0.4°C in these fish (Anttila et al., 2017). These findings support our conclusion that cold life-conditioned juvenile sea bass might be less able to cope with large acute temperature changes than their warm life-conditioned siblings.

While acute warming impairs the performance of juvenile seabass heart mitochondria, the warm life-conditioned fish showed higher mitochondrial functionality, indicating that the chosen cold life-conditioning temperature is not the optimal temperature for Atlantic juvenile seabass. The thermal biology of *D. labrax* has been the topic of several studies, although mainly on Mediterranean populations (e.g. Marangos et al., 1986; Koumoundouros et al., 2001; Lanari et al., 2002; Person-Le Ruyet et al., 2004; Dülger et al., 2012; but see Russel et al., 1996; Ayala et al., 2003; Gourtay et al., 2018). However, in contrast to the Mediterranean populations, which are exposed to higher habitat temperatures (typical annual range 13–29°C; Person-Le Ruyet et al., 2004), the Atlantic population experiences temperatures lower than 15°C for most of the year and mainly within the range 6–18°C along the coast of France up to the North Sea (Russel et al., 1996). Our fish are the offspring of fish caught in the Bay of Biscay. In these latitudes, spawning, egg development and larval hatching take place at temperatures of 8–13°C (Jennings and Pawson, 1992) and later life stages experience mainly temperatures between 6 and 18°C (Russel et al., 1996). Therefore, the temperature range we used for incubating the larvae in the cold life condition was slightly above the natural temperature range of seabass larvae from the chosen distribution area. However, for juvenile incubation the cold life condition temperature range of 15–18°C was well within the natural temperature range during summer. The temperature of the warm life-conditioned juveniles was consequently above the temperature range of their natural habitat in the Bay of Brest. Our study thus provides evidence that the seabass from the chosen population are not yet fully adapted to lower temperatures, as the warm life-conditioned juveniles displayed much better mitochondrial functionality than the cold life-conditioned animals, reflecting their evolutionary origin in warmer waters.

As a consequence of warm life conditioning, L_{Omy} and *P* respiration rates were both significantly decreased, while RCR_{Omy}

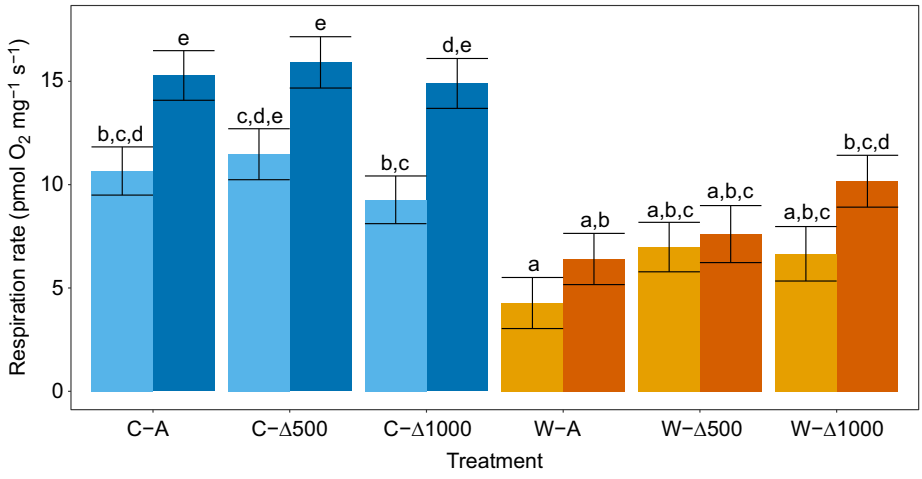

Fig. 5. LEAK respiration with oligomycin (L_{Omy}) of permeabilized heart ventricle fibers of European seabass. Data are lsmeans±s.e.m. Different letters indicate significant differences (LME, $P<0.05$); blue: cold life-conditioned fish (C), orange: warm life-conditioned fish (W), light shading: cold assay temperature, dark shading: warm assay temperature. A, ambient P_{CO_2}; Δ500, ambient+500 µatm CO_2; Δ1000, ambient+1000 µatm CO_2. $n_{C–A}=16/15$, $n_{C–Δ500}=15/16$, $n_{C–Δ1000}=16/16$, $n_{W–A}=16/15$, $n_{W–Δ500}=17/11$, $n_{W–Δ1000}=13/15$, for cold/warm assay temperature, respectively.

was significantly increased, which was the case in all treatments. The increased RCR_{Omy} in warm life-conditioned fish in comparison to cold life-conditioned fish indicates that although mitochondrial capacity was decreased in warm life-conditioned fish (decreased P), mitochondrial efficiency was increased (decreased L_{Omy} and increased RCR_{Omy}). This could translate into higher growth rates: Shama et al. (2014) found that lower P capacity resulted in optimized metabolic rate that could generate higher scope for growth in sticklebacks acclimated to warmer temperatures. Additionally, in brown trout, lower food intake and failure to grow were correlated to high L_{Omy} respiration rate and lower mitochondrial coupling in liver and muscle mitochondria (Salin et al., 2016a). In other words, individuals with more efficient oxidative phosphorylation tend to grow better than those with less efficient mitochondria. Our study reflects these findings: the warm life-conditioned juveniles showed higher RCR_{Omy} and lower P respiration rates, while being significantly larger than the cold life-conditioned fish, even when compared at equal age in degree days.

In our study, CIV or cytochrome *c* oxidase (CCO) activity was not affected by thermal life condition. As terminal electron acceptor of the electron transport system, CCO is important in aerobic respiration and was found to be the controlling site of mitochondrial respiration and ATP synthesis (Villani and Attardi, 2001; Gnaiger, 2009, 2012; Kadenbach et al., 2010). CCO generally displays excess capacity, especially in heart tissue (Gnaiger et al., 1998). In our study, CIV had excess capacity 1.5- to 2-fold higher than P respiration rate in all treatments, which is within the scope typically found in fish (1.5–3.2, Hilton et al., 2010; 1.8–2.7, Iftikar et al., 2015; 1.9–2.6, Salin et al., 2016b). Therefore CIV is not limiting the capacity of juvenile seabass mitochondria.

Conclusion

Although we used specimens originating from a northern population of seabass for this study, the results altogether indicate that the mitochondrial metabolism still supports (and favors) temperatures as found in Mediterranean specimens. Consequently, juvenile seabass in the North Atlantic might benefit from increased temperatures. Within the limits of this study, we also observed a high capacity to cope with ocean acidification, although this was less pronounced under ocean acidification and warming. The results of this study indicate that juvenile European seabass will be able to survive in an acidifying and warming ocean; however, there are further bottlenecks that may constrict their survival in a future climate. Firstly, other life stages, especially egg and larval stages, might be more vulnerable to temperature changes and increased P_{CO_2}; and secondly, other important traits, such as behavior or reproductive capacity and phenology might be affected differently by ocean acidification and warming. Consequently, other traits and life stages shall be analyzed in further studies.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.H., G.C., F.C.M.; Methodology: S.H., N.L., G.C., F.C.M.; Validation: S.H., F.C.M.; Formal analysis: S.H., G.C., F.C.M.; Investigation: S.H., L.C., N.L., G.C., F.C.M.; Resources: G.C., F.C.M.; Data curation: F.C.M.; Writing - original draft: S.H., F.C.M.; Writing - review & editing: S.H., L.C., G.C., F.C.M.; Visualization: S.H.; Supervision: G.C., F.C.M.; Project administration: F.C.M.; Funding acquisition: G.C., F.C.M.

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Data availability

Datasets of mitochondrial respiration and water conditions during rearing, as well as additional information on larval rearing are available online from PANGAEA (www.pangaea.de).

Supplementary information

Supplementary information available online at http://jeb.biologists.org/lookup/doi/10.1242/jeb.213017.supplemental

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