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RESEARCH ARTICLE

Effects of ocean acidification over successive generations decrease resilience of larval European sea bass to ocean acidification and warming but juveniles could benefit from higher temperatures in the NE Atlantic

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ABSTRACT

European sea bass (*Dicentrarchus labrax*) is a large, economically important fish species with a long generation time whose long-term resilience to ocean acidification (OA) and warming (OW) is not clear. We incubated sea bass from Brittany (France) for two generations (>5 years in total) under ambient and predicted OA conditions (P_{CO_2}: 650 and 1700 µatm) crossed with ambient and predicted OW conditions in F1 (temperature: 15–18°C and 20–23°C) to investigate the effects of climate change on larval and juvenile growth and metabolic rate. We found that in F1, OA as a single stressor at ambient temperature did not affect larval or juvenile growth and OW increased developmental time and growth rate, but OAW decreased larval size at metamorphosis. Larval routine and juvenile standard metabolic rate were significantly lower in cold compared with warm conditioned fish and also lower in F0 compared with F1 fish. We did not find any effect of OA as a single stressor on metabolic rate. Juvenile $P_{O_2,crit}$ was not affected by OA or OAW in both generations. We discuss the potential underlying mechanisms resulting in the resilience of F0 and F1 larvae and juveniles to OA and in the beneficial effects of OW on F1 larval growth and metabolic rate, but contrastingly in the vulnerability of F1, but not F0 larvae to OAW. With regard to the ecological perspective, we conclude that recruitment of larvae and early juveniles to nursery areas might decrease under OAW conditions but individuals reaching juvenile phase might benefit from increased performance at higher temperatures.

KEY WORDS: *Dicentrarchus labrax*, Performance, Multi-stressor effects, Metabolic rate, Larval growth, Juvenile growth, Teleost

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INTRODUCTION

Climate change is increasing ocean surface temperatures (ocean warming, OW), as well as decreasing ocean pH (ocean acidification, OA). OW as a single stressor on fish metabolism has been investigated intensively since the 1980s in a variety of fish species and life stages and directly influences their metabolism and therefore their growth (Johnson and Katavic, 1986; Peck, 2002; Pörtner et al., 2007) and reproduction success (for review, see Llopiz et al., 2014), as well as their distribution range and abundance (Turner et al., 2009; Pörtner, 2006). OW can increase growth rates of larval and juvenile fish (McMahon et al., 2020a; Baumann, 2019; Chauton et al., 2015), within their thermal window. Although studies on larvae are less numerous than those on adults and juveniles, it has become obvious that larvae are less resilient to OW than adults and juveniles (Dahlke et al., 2020a).

Initially, fish had been thought to be less vulnerable to OA because of their well-developed acid–base regulation systems (Heuer and Grosell, 2014), yet their capacity to cope with OA and ocean acidification and warming (OAW) as co-occurring stressors has been investigated intensively during the last decade with species- and life stage-specific results (Cattano et al., 2017): OA levels between 700 and 1600 µatm CO_2 can lead to increased larval growth (mahi-mahi: Bignami et al., 2014; clownfish: Munday et al., 2009), but decreased larval swimming performance (mahi-mahi: Bignami et al., 2014; dolphinfish: Pimentel et al., 2014) and larval metabolic rate (dolphinfish: Pimentel et al., 2014). OA also induced severe to lethal tissue damage (cod larvae: Frommel et al., 2011), decreased swimming performance, maximum metabolic rate and aerobic scope (Australasian snapper juveniles: McMahon et al., 2020b), and increased larval otolith size, with possible implications for hearing sensitivity (cobia and mahi-mahi: Bignami et al., 2013, 2014). In other species, growth was decreased by OA (inland silverside juveniles: Baumann et al., 2012), or not affected (Atlantic halibut juveniles: Gräns et al., 2014; cobia larvae: Bignami et al., 2013; Australasian snapper larvae: McMahon et al., 2020a). In some species, OA even improved performance (e.g. increased survival of Australasian snapper larvae: McMahon et al., 2020a). Dahlke et al. (2020b) showed that Atlantic cod embryos demonstrated poor acid–base regulation capacity before and during gastrulation, connected to increased mortality under OA and OAW. In contrast, acid–base regulation capacity after gastrulation was similar to that of adult cod. If the two stressors were combined, the effects became more unidirectional and were synergistic in most fish species, e.g. OAW increased growth and survival in larval and juvenile sea bass in their Atlantic populations, but decreased physiological performance (Pope

et al., 2014). The cumulative consequences of these changes are yet to be determined.

An important factor for projecting whether a species will be able to keep their distribution range under changing conditions is their potential and capacity to acclimate and adapt over generations. Few studies have so far reared fish for more than one generation or examined transgenerational effects for fish in the context of OAW, with trait- and species-specific capacities to adapt to future conditions. For example, in cinnamon anemone fish (*Amphiprion melanopus*), the negative effect of OA on escape responses was reduced in some traits if the parents were exposed to OA (Allan et al., 2014), whereas in spiny damselfish (*Acanthochromis polyacanthus*), negative effects on olfactory responses were not reduced after parental exposure to OA (Welch et al., 2014). In addition to the low number of studies on transgenerational effects, they usually used small fish, with short generation times, and applied only one stressor, either OW or OA. Little is known about the combined effect of several stressors on economically important larger-sized fish with longer generation times and thus multi-stressor, transgenerational studies on such fish are necessary to project future distribution of fish.

Consequently, in our study, we used European sea bass *Dicentrarchus labrax* as a larger, long-lived model species. Sea bass is an economically important species in industrial and recreational fishing as well as in aquaculture (160,000 tons in 2015; Bjørndal and Guillen, 2018). Sea bass can reach an age of 24 years in the Atlantic population (Irish waters; Kennedy and Fitzmaurice, 1972). Although they are generally rather resilient towards environmental fluctuations, effects of OW and OA have been reported for several seabass life stages: OW increased growth rate in larval sea bass, although at the expense of decreased swimming performance (Atlantic population, 15–20°C; Cominassi et al., 2019). Exposure to OA throughout larval development increased mineralization and reduced skeletal deformities (Atlantic population, 19°C and 15 and 20°C, respectively; Crespel et al., 2017; Cominassi et al., 2019). In combination, OAW did not have additional effects on larval growth, swimming ability and development from those already observed separately (Atlantic population; Cominassi et al., 2019). Juvenile sea bass are highly tolerant to temperature (Dalla Via et al., 1998; Claireaux and Lagardère, 1999) and show some degree of tolerance to OA as a single stressor at the mitochondrial level (Atlantic population; Howald et al., 2019). The effect sizes of OA and OW are different: OW as a single stressor increased growth and digestive efficiency, while OA did not affect these traits. The two stressors combined caused reduced growth and digestive efficiency compared with the impact of OW alone. Low food ratios enhanced this effect, resulting in an even more pronounced growth and digestive efficiency reduction than under OAW alone (Atlantic population; Cominassi et al., 2020).

This study aimed to investigate the effect of OAW as well as the effect of OA over two successive generations (F0 and F1) on larval and juvenile growth and metabolism. Therefore, we incubated sea bass from an Atlantic population for two generations (>5 years in total) under current and predicted OA conditions (P_{CO_2}: 650 and 1700 µatm) and applied a warming condition on larvae and juveniles of the F1 generation (ambient, 15–18°C; and Δ5°C, 20–23°C). To study the effect of OA (F0, F1), OW (F1) and OAW (F1) on sea bass, we investigated growth (F0, F1) through ontogeny as a proxy for whole-organism fitness. In addition, we measured routine metabolic rate (RMR; F1) of larvae, as well as standard metabolic rate (SMR; F0, F1) and critical oxygen concentration ($P_{O_2,crit}$; F0, F1) of juvenile sea bass, to unravel the underlying mechanisms resulting in possible growth differences. In F0, no effect of OA on larval and juvenile growth or juvenile SMR and $P_{O_2,crit}$ was found (Crespel et al., 2017, 2019). Those traits were compared in F0 and F1 fish to determine the effects due to parental acclimation to different OA levels. Our hypotheses were: (1) OW will lead to increased growth and metabolic rate in F1 larvae and juveniles; (2) OA alone will not have significant effects on larval and juvenile growth and metabolism in F1, as sea bass seem to be quite tolerant to OA and no detrimental effects were found in F0; and (3) in combination, OA will lead to synergistic OAW effects, reflected in lower growth in larvae and juveniles.

MATERIALS AND METHODS

The present work was performed within the facilities of the Ifremer-Centre de Bretagne (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) (authorizations APAFIS 4341.03, #201620211505680.V3 and APAFIS 14203-2018032209421223 for F0 and F1, respectively).

Animals and experimental conditions

Sea bass, *Dicentrarchus labrax* (Linnaeus 1758), were reared from early larval stage onwards in two OA treatments in F0 and four OAW treatments in F1. A flow chart summarizing temperature and P_{CO_2} conditions as well as replicate tank number, tank volume and number of individuals per tank is shown in Fig. 1; the timeline for fish rearing is shown in Fig. S1. F0 fish were reared in two OA scenarios, following the predictions of the Intergovernmental Panel on Climate Change (IPCC, 2021) for the next 130 years: today's ambient situation in coastal waters of Brittany and the Bay of Brest (A, ∼650 µatm; see Pope et al., 2014; Duteil et al., 2016) and a scenario according to SSP5-8.5, projecting a ΔP_{CO_2} of 1000 µatm (Δ1000, ∼1700 µatm). Adults from these two treatments were used in the reproduction experiments to generate F1. Sea bass of F1 were reared under the same OA conditions as their respective parents. Additionally, two different temperatures were applied on each OA condition in F1 to create a cold (C) and a warm (W) life condition scenario or four OAW conditions (C-A, C-Δ1000, W-A and W-Δ1000), respectively. As larvae and post-larval juveniles would display different growth rates under the different life condition scenarios, we adopted the concept of degree days [dd=dph×temperature (T in °C)] as a basis for comparison between them. This concept allows comparison of fish at their physiological age rather than their chronological age and has been shown to be an effective way of normalizing growth at different temperatures (Peck et al., 2012).

Larval rearing was performed in a temperature-controlled room and water temperature was fixed to 19°C in F0, and 15 and 20°C in F1 C and W, respectively. In juveniles and adults, water temperature of F0 and F1 C sea bass was adjusted to ambient temperature in the Bay of Brest during summer (up to 19°C), but was kept constant at 15 and 12°C for juveniles and adults, respectively, when ambient temperature decreased below these values. The F1 W was always 5°C warmer than the F1 C treatment.

During larval rearing, the photoperiod was set to 24 h darkness during the first week and 16 h light and 8 h darkness (12 h each in F1) every day afterwards. Light intensity increased progressively during the larval rearing period from total darkness to about 100 lx (Table S1). To work in the larval rearing facilities, headlamps were used (set to the lowest light intensity). In the juvenile and adult

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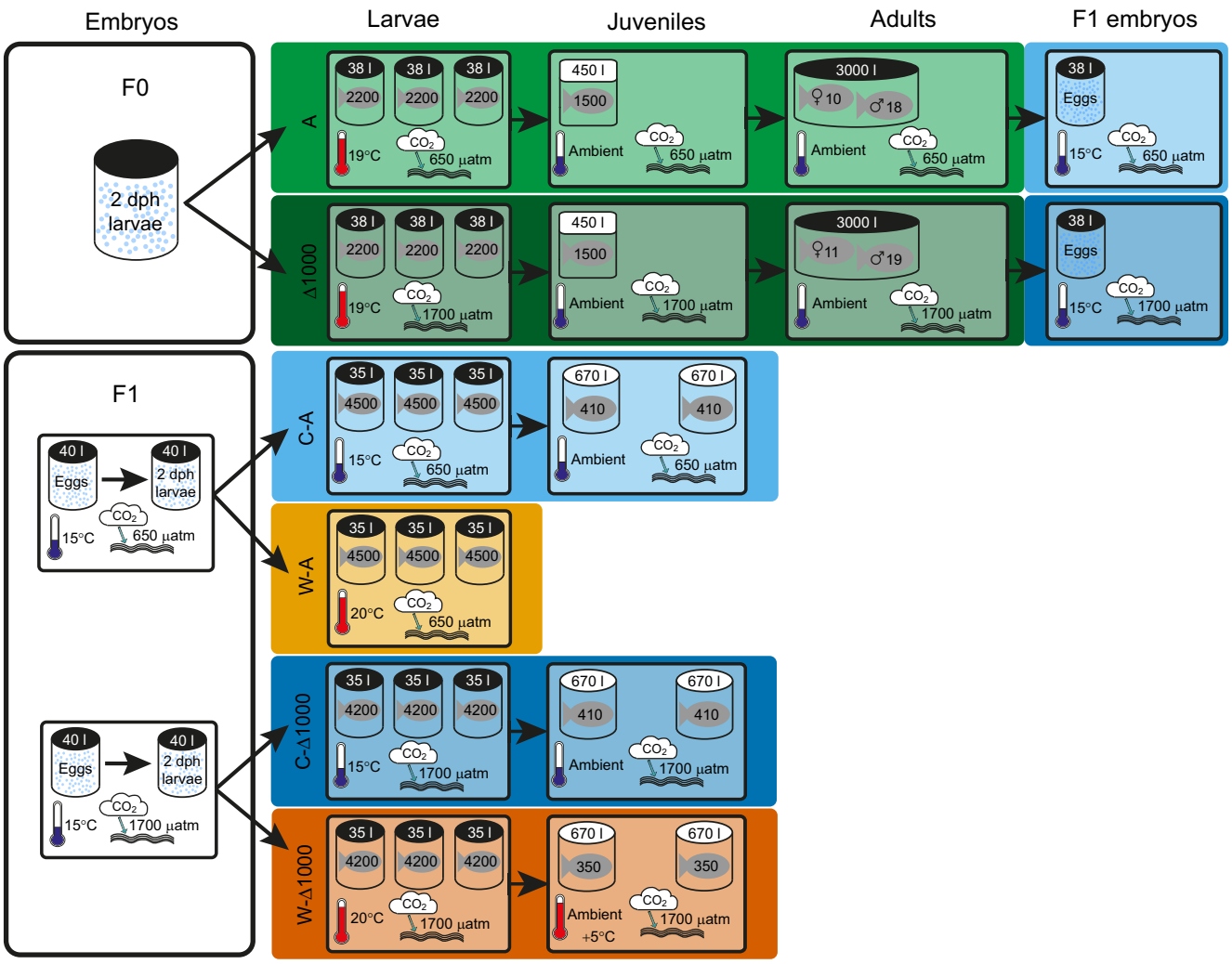

Fig. 1. Schematic overview summarizing the rearing conditions of two generations of sea bass under different ocean acidification–warming (OAW) scenarios. Rearing conditions in the experiments on two successive generations of European sea bass. Parental (F0) and offspring (F1) generations were raised and reared under two acidification conditions: today's ambient levels in the Bay of Brest (650 µatm; A) and a projected condition following the worst-case scenario prediction of the IPCC (2021) (1700 µatm; Δ1000). In F1, two temperatures were applied to each of the two acidification conditions: a cold life condition (C) with 15°C during the larval phase and ambient conditions during the summers (up to 18°C), and a warm life condition (W) with 20°C during the larval phase and ambient +5°C during summers. Age of larvae (days post-hatching, dph), tank volume, number of larvae/adults per tank, temperature and P_{CO_2} are indicated.

rearing facilities, photoperiod followed natural conditions (adjustment once a week).

F0 generation

Larval rearing

F0 larval rearing and origin is described in detail in Crespel et al. (2017, 2019), briefly, larvae were obtained from the aquaculture facility Aquastream (Ploemeur-Lorient, France) at 2 dph (October 2013). F0 larvae were randomly distributed among the two OA conditions described above. Larvae were reared in nine black 38 l tanks initially stocked with *ca*. 2200 larvae per tank in triplicate for all conditions. Larvae were fed *ad libitum* via continuous delivery of *Artemia* nauplii until 28 dph. Afterwards, commercial dry pellets (Neo Start, LeGouessant, France) were fed for the rest of the larval period.

Juvenile rearing

Juvenile rearing is described in detail in Crespel et al. (2019). Briefly, the early juveniles were counted per tank and transferred from larval to juvenile rearing facilities at ∼820 dd (45 dph). Juveniles of one condition were combined and kept in square 450 l tanks (n=1500 fish per condition). At 8 months (about 250 dph), juveniles were PIT tagged (i.e. marked with passive integrated transponders). Juveniles were fed daily with commercial fish food (Neo Start), which was adjusted in size and amount, as recommended by the supplier (Le Gouessant, Lamballe, France). Food ratios were adjusted after each sampling for growth, approximately every 30 days or 3–4 weeks in F0 and F1, respectively (see below), using the formulae provided by Le Gouessant. Daily food ratios were supplied to the tanks by automatic feeders during the daytime.

Adult rearing

During the reproductive season in 2017 (fish were 3.5 years old), sex steroid plasma concentration was measured regularly in all adult F0 fish. The individuals with the highest concentrations were kept in round black tanks with a volume of 3 m^3 and a depth of 1.3 m. Each of the two tanks (one for each condition) was stocked with 22 males and 11 females, resulting in fish density of 11.6 kg m^{-3} and 11.0 kg m^{-3} in A and Δ1000, respectively. Mass and length were regularly measured and commercial fish food was adjusted

accordingly. Fish were fed Vitalis CAL (Skretting, Norway) during the reproduction season and Vitalis REPRO (Skretting, Norway) during the rest of the year. Vitalis REPRO was supplied to the tanks with automatic feeders during the daytime. Vitalis CAL was supplied to the tank manually in three to four rations on week days.

F1 generation

Embryos were obtained by artificial reproduction of F0 fish. Briefly, once the water temperature reached 13°C and the first naturally spawned eggs were observed in the egg collectors, females were injected with gonadotropin-releasing hormone (GnRH, 10 µg kg^{-1}) to accelerate oocyte maturation (23 March 2018). After 3 days (26 March 2018), eggs and milt were stripped from ripe females and males, respectively, and artificial fertilization was performed following the protocol of Parazo et al. (1998). Briefly, eggs (10 ml l^{-1}) were mixed with seawater and milt (0.05 ml milt l^{-1} seawater). Ten females (1.56±0.24 kg) were crossed with 18 males (1.07±0.16 kg) and 11 females (1.28±0.30 kg) were crossed with 19 males (0.99±0.19 kg) in the A and Δ1000 groups, respectively. Fertilized eggs were incubated in 40 l tanks (without replicates) at 15°C and under the same P_{CO_2} conditions as the respective F0. Hatching occurred after 4 days (30 March 2018).

Larval rearing

Two days after hatching (2 April 2018), larvae were distributed into 12 black 35 l tanks. Triplicate tanks were allocated to each of the four OAW treatments with *ca*. 4500 and 4200 larvae per tank in A and Δ1000 tanks, resulting in a total of *ca.* 13,500 and 12,800 larvae per condition in A and Δ1000, respectively. The temperature of the tanks allocated to the warm life condition was increased stepwise by 1°C day^{-1} during the following 5 days. 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 seawater. Larvae were fed *ad libitum* with artemia during the day; excess artemia left the tank via the waste water outflow. Larval mortality was 26–96%, without any pattern for the OAW condition (Table S2). High mortality of sea bass larvae, especially during early larval rearing, is common in science and aquaculture (e.g. Nolting et al., 1999; Suzer et al., 2007; Villamizar et al., 2009). We could not find any signs of infection either in the tanks with high mortality or in the tanks with lower mortality rates. However, as larval mortality was unreasonably high (96%) within the first week in one of the replicate tanks of the W-A treatment, remaining larvae in this tank were euthanized (sedation with eugenol followed by an overdose of MS-222) and not used for further analysis. The water surface was kept free of oily films using a protein skimmer. Water exchange was set to 25 l h^{-1} and increased stepwise to 40 l h^{-1} at the end of larval rearing.

Juvenile rearing

At ∼950 dd, the early juveniles were counted per tank and transferred from larval to juvenile rearing conditions (48 dph, 17 May 2018 and 63 dph, 01 June 2018 for W and C, respectively). For F1 W, only the Δ1000 fish were transferred to juvenile rearing facilities. Juveniles were randomly allocated to duplicate tanks per condition. A swim bladder test was done at 1680 dd (83 dph, 21 June 2018) and 1661 dd (104 dph, 12 July 2018) for F1 W and F1 C, respectively. Briefly, the fish were anaesthetized and introduced into a test container with a salinity of 65 psu (Marine SeaSalt, Tetra, Melle, Germany). In F1 W, all floating fish with a developed swim bladder were counted and kept in the rearing tanks, resulting in 355 fish per tank (710 fish in total). In F1 C, 410 fish per tank were randomly selected (820 fish per condition), to have similar stocking densities in W and C. Non-floating fish as well as excess F1 C fish were counted and euthanized (sedation followed by an anaesthetic overdose). The juveniles were reared in round tanks with a volume of 0.67 m^3 and a depth of 0.65 m. During the first 5 days after moving to juvenile rearing, the juveniles were fed *Artemia* nauplii and commercial fish food. Afterwards, commercial fish food was fed as described above.

Experimental conditions

Seawater preparation

The seawater 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), degassed using a column packed with plastic rings, filtered using a 2 µm membrane and finally UV sterilized (PZ50, 75 W, Ocene) assuring high water quality.

Water conditions for the rearing tanks were preadjusted to the desired OAW condition in header tanks. Seawater arrived in a reservoir next to the rearing facilities, after passing the tungsten heater; in F1, two different reservoirs were used to create the different temperature conditions. The temperature-controlled water supplied the header tanks within the rearing facilities to adjust the water to the desired OA condition. Each header tank supplied water to all replicate tanks of the respective condition.

In F0 larvae and juveniles, the water pH in the header tank was controlled by an automatic injection system connected to a pH electrode (pH Control, JBL), which injected either air (A) or CO_2 (Δ1000), to control water pH. For the Δ1000 F1 larvae, the CO_2 bubbler was installed in the middle of the header tank and the water was mixed continuously with a pump. CO_2 bubbling was adjusted by a flow control unit, when pH deviated from the desired value.

Older F0 A juveniles (>2 years) and adults, as well as F1 A larvae and juveniles received water directly from the respective reservoir, without header tank. Additionally, as water exchange rates became too high for the automatic injection system and the header tank, PVC columns were installed to control the pH in the rearing tanks. The temperature-controlled water arrived at the top of the column and was pumped from the bottom of the column to the rearing tanks. The CO_2 bubbler was installed at the bottom of the column and was adjusted by a flow control unit, when pH deviated from the desired value.

Calculation of water chemistry

The Microsoft Excel macro CO2sys (Lewis and Wallace, 1998) was used to calculate seawater carbonate chemistry; the constants after Mehrbach et al. (1973; as cited in CO2sys) refitted by Dickson and Millero (1987; as cited in CO2sys) were employed.

From October 2015 onwards (late juveniles of F0), total alkalinity was measured 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 (mol l^{-1}) was then calculated with the following formula:

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

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

Table 1. Water parameters during the F0 larval and early juvenile phase of European sea bass

Phase	Treatment	pH_{NBS}	pH_{total}	Temperature (°C)	Salinity (psu)	TA (µmol l^{-1})	P_{CO_2} (µatm)	PO_4^{3-} (µmol l^{-1})	SiO_4 (µmol l^{-1})
Larvae	A	7.96±0.01	7.89±0.01	19.2±0.3	33.8±0.2	2294±3	589±10	0.57±0.01	8.94±0.06
	Δ1000	7.59±0.00	7.54±0.03	19.2±0.3	33.8±0.2	2306±9	1521±97	0.57±0.01	8.94±0.06
Juveniles	A	8.05±0.01	7.94±0.03	15.3±0.1	34.3±0.2	2294±10	516±31	0.71±0.08	8.35±0.26
	Δ1000	7.61±0.01	7.53±0.02	15.3±0.1	34.3±0.2	2280±16	1489±42	0.71±0.08	8.35±0.26

The larval period lasted until 45 days post-hatching (dph; ∼900 degree days, dd); the early juvenile period lasted until 1.5 years. Data are means±s.e.m. over all measurements per condition (triplicate tanks for larvae, single tanks for juveniles). Temperature and pH (NBS scale) were measured daily. pH (total scale), salinity, phosphate, silicate and total alkalinity (TA) were measured once at the beginning and once at the end of the larval phase and 9 times during the juvenile phase; P_{CO_2} was calculated with CO2sys. A, ambient P_{CO_2}; Δ1000, ambient+1000 µatm CO_2 (see Crespel et al., 2017; Crespel et al., 2019).

the sample (l), H^+ is hydrogen activity (10^{-pH}) and γ^{H+} is the hydrogen activity coefficient (here γ^{H+}=0.758).

Water quality control

Temperature and pH were checked each morning with a handheld WTW 330i or 3110 pH meter (Xylem Analytics Germany, Weilheim, Germany; with electrode: WTW Sentix 41, NIST scale) before feeding the fish. The pH meter and the automatic injection system were calibrated weekly with fresh buffers (Merck, Germany) until F0 juveniles reached 2 years. Measured values never differed more than 2% from the target values. Afterwards, the pH meter was calibrated daily with NIST certified WTW technical buffers pH 4.01 and pH 7.00 (Xylem Analytics Germany).

Total pH was determined twice during F0 larval rearing (start and end) and 9 times during F0 juvenile rearing following Dickson et al. (2007) using *m*-cresol purple as an indicator. Additionally, water samples were sent to LABOCEA (France) to measure total alkalinity by titration, as well as phosphate and silicate concentration by segmented flow analysis following Aminot et al. (2009).

In later F0 juveniles (>2 years) and adults as well as F1 larvae and juveniles, total alkalinity was measured monthly or weekly in F0 and F1, respectively, following the protocol described above. Oxygen saturation (WTW Oxi 340, Xylem Analytics Germany) and salinity (WTW LF325, Xylem Analytics Germany) were measured together with total alkalinity (monthly in F0 and weekly in F1). The tanks were cleaned daily after pH measurements. Water flow within the tanks was adjusted once a week, so that oxygen saturation levels were kept >85%, with equal flow rates in all tanks of one temperature. All water parameters are summarized in Table 1 for F0 larvae and juveniles and Table 2 for F0 adults (2 years before spawning) and F1 larvae and juveniles.

Growth

Larval growth

F0 larvae

Larval growth was measured as described in Crespel et al. (2017). Briefly, 10 larvae per tank were sampled each week, starting at 15 dph and ending at 45 dph, when 30 larvae per tank were sampled. For growth measurements, larvae were anaesthetized with phenoxyethanol (200 ppm) and their wet mass (WM), as well as body length (BL) were measured. BL in F0 larvae was measured with a calliper from the tip of the snout to the end of the notochord until flexion; afterwards, fork length was considered as BL (see Fig. S2).

F1 larvae

In F1 larvae, individuals were sampled every 200 dd from 100 to 900 dd to follow growth throughout the larval phase. At each sampling point, 20 larvae per tank were anaesthetized with MS-222 (50 mg l^{-1}, Pharma Q) prior to feeding and directly photographed individually with a microscope (Leica M165C). The larvae were then frozen in liquid nitrogen and stored at −80°C until dry mass (DM) measurements. The software ImageJ (Schneider et al., 2012) was used to determine BL of larvae (see Fig. S2 for the definition of BL).

Juvenile growth

BL and WM were measured approximately every 30 days in F0 and every 3–4 weeks in F1 juveniles. Early juveniles were starved for 1 day prior to growth sampling. Later on, this was increased to 2 days, to make sure that digestive tracts were empty. Juveniles were caught from their tanks and anaesthetized with MS-222 (Pharma Q). The concentration of anaesthetic was adjusted to reach a loss of equilibrium within <5 min, typically 0.2 g l^{-1}. WM and BL were

Table 2. Water parameters in the 2 years before spawning of F0 (2016–2018) and during the F1 larval and juvenile phase of European sea bass

Phase	Treatment	pH_{free}	Temperature (°C)	Salinity (psu)	O_2 (% air saturation)	TA	P_{CO_2} (µatm)
F0	A	7.95±0.02	14.1±0.6	33.6±0.3	92.4±1.7	2406±49	670±40
	Δ1000	7.59±0.02	14.1±0.6	33.6±0.3	92.4±1.9	2411±46	1616±74
F1 larvae	C-A	8.06±0.01	15.3±0.1	31.8±0.1	94.3±1.0	2360±23	504±19
	C-Δ1000	7.53±0.01	15.5±0.1	31.8±0.1	94.3±0.8	2330±22	1872±74
	W-A	7.96±0.01	20.2±0.2	31.7±0.0	84.9±3.4	2311±32	656±22
	W-Δ1000	7.61±0.01	20.2±0.2	31.8±0.0	88.1±1.7	2321±32	1624±59
F1 juveniles	C-A	7.94±0.01	16.1±0.2	33.0±0.1	92.4±0.5	2376±15	696±19
	C-Δ1000	7.60±0.01	16.3±0.2	33.0±0.1	94.3±0.5	2380±14	1603±32
	W-A	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	W-Δ1000	7.57±0.02	22.7±0.2	33.0±0.2	86.3±1.3	2323±16	1866±83
	SW	8.07±0.01	15.0±0.5	34.6±0.3	101.0±0.8	2441±23	609±37

The larval period lasted until 48 dph (∼900 dd) and 63 dph (∼900 dd) for the warm and cold life condition, respectively; the juvenile period lasted until 180 dph (∼4000 dd) and 319 dph (∼5100 dd) for warm and cold conditioned fish, respectively. Data are means±s.e.m. over all replicate tanks per condition. Temperature, pH, salinity, oxygen and total alkalinity (TA) were measured weekly in F1 and monthly in F0; P_{CO_2} was calculated with CO2sys; seawater (SW) measurements were conducted in 2017 and 2018. A, ambient P_{CO_2}; Δ1000, ambient+1000 µatm CO_2; C, cold life condition; W, warm life condition.

directly determined with a precision balance (Sartorius MC1 AC210P) and callipers. For all sampling, only the morning hours were used, to avoid diurnal artefacts in data.

Data handling

For F1 larvae and juveniles, mean specific growth rate (SGR, % day^{-1}) of each tank was calculated after Sutcliffe (1970) with the following formula:

$$\mathrm{SGR} = 100\ (e^{g} - 1). \quad (2)$$

The instantaneous growth coefficient (g) was calculated as:

$$g = \frac{\ln S_1 - \ln S_0}{\Delta t}, \quad (3)$$

where S_0 and S_1 are initial and final size (BL, WM or DM) and Δt is the time between the two measurements (days). Initial and final sizes were calculated for three quantiles (0.05, 0.5 and 0.95) for each tank ('ecdf' function in R).

Q_{10} was calculated with the following formula:

$$Q_{10} = \left(\frac{\mathrm{SGR_W}}{\mathrm{SGR_C}}\right)^{\left(\frac{10}{T_\mathrm{W} - T_\mathrm{C}}\right)}, \quad (4)$$

where SGR is specific growth rate, T is temperature, and subscripts W and C represent the W and C condition.

Respirometry

F1 larvae

Larval respiration measurements were conducted from approximately 350 to 950 dd in all conditions (18–47 dph and 25–63 dph in W and C, respectively).

Larval respiration was measured in an intermittent flow system. The setup consisted of up to eight 4 ml micro-respiration chambers with a glass ring (Unisense A/S, Aarhus, Denmark), equipped with a glass-coated magnetic stirrer (Loligo® Systems, Viborg, Denmark) and a stainless steel mesh (Loligo® Systems), to separate the stirrer from the larva. The magnetic stirrers were connected to one stirrer controller (Rank Brothers Ltd, Cambridge, UK). The chamber was closed with a custom-made glass lid with three metal ports: two with a diameter of 0.8 mm for water inflow and outflow during flushing, and one with a diameter of 1.2 mm to insert the oxygen sensor into the chamber. Oxygen concentration within the chamber was measured with oxygen microsensors connected to a FireSting oxygen meter (PyroScience GmbH, Aachen, Germany). The respiration chambers were placed within a rack without shielding between the individual chambers. The rack holding the respiration chambers was fully submerged in a water reservoir, which received flow-through water from the respective header tanks of the larval rearing. Water conditions within the water reservoir were kept at 15.5±1.5 and 21.2±1.0°C for W and C larvae, combined with the OA condition of the origin tank of the respective larvae. The reservoir was a black container, which shielded the respiration setup from external disturbance. During the flushing periods, water from the reservoir was pumped into the respiration chambers using computer-controlled flush pumps (Miniature DC pump, Loligo® Systems), relays and software (AquaResp, Copenhagen, Denmark). Four chambers were connected to one flush pump and controlled by one computer. Oxygen microsensors were calibrated to 0% saturation (nitrogen purged seawater) and 100% saturation (fully aerated seawater) prior to each measurement.

Respiration measurements were done in the larval rearing facilities with the same light conditions as for larval rearing. Larvae were fasted at least 3 h prior to respiration measurements to minimize the effect of specific dynamic action (SDA) on metabolic rate. Preliminary tests with measurements overnight showed that oxygen consumption during the 12 h after the 3 h fasting period was similar, suggesting no contribution of SDA and thus that the 3 h fasting period was sufficient for our setup. Larvae were individually placed in the respiration chambers. Oxygen partial pressure was measured every second for ∼4 h. Cycles were composed of 420 s flush, followed by 60 s wait time (time after flush pump stopped to wait for a stable drop in oxygen concentration) and 600 to 180 s measurement time (13–20 cycles per larvae). Measurement time was decreased with increasing larval size. Oxygen concentration was restored to normoxia during the flush time of each cycle and was usually kept above 75% air saturation. Background respiration was measured for 30 min (one slope) after 11 and 18 measurements in F1 C and F1 W larvae, respectively. The mean bacterial respiration was calculated for each temperature treatment and subtracted from total respiration of all larvae of this temperature to obtain oxygen consumption of the larva. Background respiration was typically 0.5–6% of total respiration. Only declines in oxygen concentration displaying R^2>0.80 were used for analysis. After the measurement, larvae were checked to make sure they were alive, anaesthetized with MS-222 (50 mg l^{-1} Pharma Q), photographed individually and frozen in liquid nitrogen. Length and DM of the larvae were obtained as described above (see Table S3). After each experiment, the respiration system was rinsed with fresh water and allowed to dry. For disinfection, the respiration chambers, the tubing of the flush pump and the oxygen sensors were additionally rinsed with ethanol, which was allowed to sit in the chambers and the tubing for at least 30 min followed by rinsing with distilled water.

Juveniles

Setup for F0 juveniles

Measurements on 15 month old F0 juveniles (F0 old) were as described in Crespel et al. (2019); measurements on 5 month old F0 juveniles (F0 young) were done similarly – and differences are indicated in parentheses. Briefly, F0 juvenile respiration was measured individually in one of four (eight) intermittent flow respirometry chambers with a volume of 2.1 l (60 ml), which were submerged in a tank that received flow-through seawater at 15±0.25°C and the respective acidification condition. The water was recirculated within the chamber with a peristaltic pump with gas-tight tubing. The oxygen probe (FireSting oxygen meter, PyroScience GmbH or multichannel oxygen meter, PreSens Precision Sensing GmbH) was placed within the recirculation loop. Oxygen sensors were calibrated to 0% saturation (sodium sulphite, saturated) and 100% saturation (fully aerated seawater) prior to each experiment. The flush pumps were controlled by relays and software (AquaResp, Copenhagen, Denmark). The setup was placed behind a curtain to avoid disturbance. Background respiration was measured after each experiment and estimated for the whole experiment by linear regression assuming zero background respiration at the beginning of the run as the entire system was disinfected with household bleach between each trial.

Setup for F1 juveniles

F1 juvenile respiration was measured in an intermittent flow system. The setup consisted of up to eight 450 ml custom-made respiration chambers. The chambers were made from Lock&Lock glass containers with plastic lid. Four rubber ports were placed into the

lid: two for water inflow and outflow during flushing cycles and two to connect the chamber to a mixing pump (Miniature DC pump, Loligo® Systems). Oxygen concentration was measured with robust oxygen probes placed within the circulation loop and connected to a FireSting oxygen meter (PyroScience GmbH) or to a multichannel oxygen meter (PreSens Precision Sensing GmbH). The respiration chambers were fully submerged in a flow-through water reservoir. Water conditions within the water reservoir were kept at 14.9±1.0 and 22.3±1.8°C for C and W larvae, combined with the OA condition of the origin tank of the respective juvenile. During the flushing periods, water from the reservoir was pumped into the respiration chambers using computer-controlled flush pumps (EHEIM GmbH & Co. KG, Deizisau, Germany), relays and software (AquaResp). Four chambers were connected to one flush pump and controlled by one computer, running either the FireSting or the PreSens oxygen meter. The setup was covered with black foil to avoid disturbance. Oxygen sensors were calibrated to 0% saturation (nitrogen purged seawater) and 100% saturation (fully aerated seawater) prior to each experiment. Background respiration was measured for 30 min (one slope) after each measurement and the run was discarded, if background respiration was >10%. After each experiment, the whole system excluding the oxygen sensors was disinfected with household bleach or Virkon® (Antec International Limited, Suffolk, UK) and rinsed with freshwater afterwards.

Measurement protocol

Respiration measurements of F0 juveniles were done on approximately 5 month old (119–165 dph) and 15 month old (454–495 dph) juveniles. F1 juvenile respiration measurements were conducted from 2900 to 3900 dd (137–178 dph, 5 months) and 4700 to 5100 dd (291–318 dph, 10 months) for F1 W and F1 C, respectively. F1 C fish were older than F1 W fish at the measurement time in order to have comparable fish sizes (see Table S4).

Juvenile sea bass were fasted for 48–72 h prior to respiration measurements to minimize the effect of residual SDA (Dupont-Prinet et al., 2010). Juveniles were randomly taken from their tank and placed individually in the respiration chambers. The whole setup was shielded from external disturbance with curtains or black foil, but the individual respiration chambers were not shielded from each other. F0 juveniles were chased until exhaustion prior to introduction into the chambers (maximum metabolic rate data partly given in Crespel et al., 2019). Each experiment lasted for about 70 h in F0 and 65 h in F1. Oxygen partial pressure was measured every second and was usually kept above 80%, until the start of the $P_{O_2,crit}$ trial (see below). Each cycle was composed of 360 s (F0) and 540 s (F1) flush time, during which oxygen concentration was restored to normoxia (until $P_{O_2,crit}$ trial), followed by 30 s wait and 210 s (F0) and 180 s (F1) measurement time. In F0, only the measurements taken after the fish fully recovered from chasing stress were used to calculate SMR, usually after 10 h. In F1, the first 5 h of each experiment were not used for analysis of SMR, to account for acclimation of the fish to the respirometer and recovery from handling stress, resulting in approximately 390 and 310 cycles in F0 and F1 juveniles, respectively. Analyses were performed only on declines in oxygen concentration displaying R^2>0.85 and R^2>0.90 in F0 and F1, respectively. On the third morning, a $P_{O_2,crit}$ trial was done on F0 old and F1 juveniles, see below. After finishing the trial or the respiration measurement for F0 young, fish were removed from the chamber. Juveniles were weighed and BL was measured prior to the experiment for F0 and after the experiment for F1. F0 old juveniles were identified by their PIT tag and returned to their origin tank after the experiment. F0 young juveniles and F1 juveniles were killed by a cut through the spine after the experiment.

$P_{O_2,crit}$ trial

On the third morning, oxygen concentration in the tank surrounding the chambers was continuously decreased, in F0 old by passing the water through a gas equilibration column supplied with nitrogen gas before pumping it into the tank. In F1, the decrease in oxygen concentration was done by bubbling nitrogen directly into the surrounding water bath. The decrease lasted over a period of 4–6 h hours to determine $P_{O_2,crit}$. When the fish lost equilibrium in the oxygen-depleted chambers, they were removed from their chamber and treated as described above.

Data handling

In F0 juveniles, the metabolic rate (MR, in mg O_2 h^{-1} kg^{-1} wet mass in F0) was calculated using AquaResp software. In F1, oxygen concentration was converted from % air saturation to nmol l^{-1} and mmol l^{-1} in larvae and juveniles, respectively ('conv_O2' function of the 'respirometry' package; https://CRAN.R-project.org/package=respirometry). MR was calculated from the raw data with the following formula:

$$\mathrm{MR} = \mathrm{Slope}\ V_{resp}, \quad (5)$$

where slope is the oxygen decline in the respiration chamber during one measurement cycle (nmol O_2 l^{-1} h^{-1} and mmol O_2 l^{-1} h^{-1} for larvae and juveniles, respectively), and V_{resp} is the volume of the respirometer (l).

RMR of F1 larvae was calculated as the mean MR throughout the measuring period (~4 h). SMR of F0 juveniles was calculated following the protocol of Chabot et al. (2016) as described in Crespel et al. (2019). SMR of F1 juveniles was calculated in R with the 'calcSMR' function of the 'fishMO2' package (http://github.com/denis-chabot/fishMO2), derived from this protocol. Briefly, the best SMR was chosen as described in Chabot et al. (2016) as either the SMR derived from the mean of the lowest normal distribution (MLND) method (SMR_{MLND}) or the SMR derived from the quantile method with P=0.2 (SMR_{quant}). SMR_{MLND} was used when the coefficient of variation (CV) was <7% or <5.4%, in F0 and F1, respectively; otherwise, SMR_{quant} was applied. Both RMR and SMR were divided by fish mass (resulting in RMR_{Raw} and SMR_{Raw}) and then corrected for allometric scaling with the following formulas:

$$\mathrm{RMR} = \mathrm{RMR}_{\mathrm{Raw}} \left(\frac{\mathrm{DM}}{\mathrm{DM}_{\mathrm{mean}}}\right)^{1-\mathrm{coeff}_{\mathrm{Larvae}}}, \quad (6)$$

$$\mathrm{SMR} = \mathrm{SMR}_{\mathrm{Raw}} \left(\frac{\mathrm{WM}}{\mathrm{WM}_{\mathrm{mean}}}\right)^{1-\mathrm{coeff}_{\mathrm{Juv}}}, \quad (7)$$

where RMR_{Raw} and SMR_{Raw} are RMR (nmol O_2 μg DM^{-1} h^{-1}) and SMR (mmol O_2 kg WW^{-1} h^{-1}) calculated as described in the text, DM is larval dry mass (μg), WM is juvenile wet mass (kg), DM_{mean} and WM_{mean} are mean DM and WM of all larvae and juveniles, respectively, and $coeff_{Larvae}$ and $coeff_{Juv}$ is the allometric scaling coefficient for larvae (0.89) and juveniles (0.99), respectively. The allometric scaling coefficients used were the slopes of linear regressions of MR over mass in the whole larval (F1) and juvenile (F0 and F1 together) dataset. Q_{10} was calculated with the same formula as used for SGR (see Eqn 4).

$P_{O_2,crit}$ was calculated with the 'calcO2crit' function of the 'fishMO2' package (http://github.com/denis-chabot/fishMO2), or according to Claireaux and Chabot (2016).

Statistical analysis

All statistics were performed with R (http://www.R-project.org/). All data were tested for outliers (Nalimov test), normality (Shapiro–Wilk's test) and homogeneity (Levene's test). None of the datasets met the assumptions for ANOVA; therefore, all data were fitted to linear mixed effects models (LME models, 'lme' function of the 'nlme' package; https://CRAN.R-project.org/package=nlme). Rearing tank was included as a random effect in all models. For the respirometry experiments, respirometer was also included as random effect. 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 lowest Akaike information criteria (AIC) values. After fitting fixed and random effects, a backwards model selection process was applied to determine the significant and fixed variables and interactions. 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 were produced from the lsmeans-data with the 'ggplot2' package (Wickham, 2016). All data are shown as lsmeans±s.e.m.

Growth data

Larval BL (F0 and F1 larvae)

Larval BL at mouth opening was only measured in F1 larvae. As these were reared in a full-factorial design, temperature condition, P_{CO_2} concentration and their interactions were included as fixed effects in the model. Across generations, the dataset for larval BL at metamorphosis and over time was imbalanced; therefore, it was not possible to test the effect of temperature, P_{CO_2} condition, generation and their interaction separately. Instead, treatment was used as fixed variable in the model for larval BL at metamorphosis, resulting in six groups: F0 A, F0 Δ1000, F1 C-A, F1 C-Δ1000, F1 W-A and F1 W-Δ1000. For larval BL over time, treatment, age and the interaction between group and age were included as fixed effects in the model.

Larval DM (F1 larvae)

Larval DM was only measured in F1 larvae; therefore, temperature condition, P_{CO_2} concentration, age and their interactions were included as fixed effects in the model for log-transformed larval DM over time. Larval DM at mouth opening and metamorphosis was analysed with temperature condition, P_{CO_2} concentration and their interactions as fixed effects.

Juvenile BL and WM over time (F1 juveniles)

As F0 and F1 juveniles had different temperature life histories as well as rearing conditions, their growth rates over time were not directly compared. Because of an imbalanced dataset in F1 juveniles, it was not possible to test the effect of temperature, P_{CO_2} condition and their interaction separately. Instead, as for larval BL, treatment was used as fixed variable, resulting in three groups: F1 C-A, F1 C-Δ1000 and F1 W-Δ1000. Treatment, age and the interaction between treatment and age were included as fixed effects in the models for juvenile BL and log-transformed juvenile WM over time.

Juvenile BL and WM at 3000 dd (F0 and F1 juveniles)

Juvenile BL and WM were compared at 3000 dd across generations. Because of the imbalanced dataset, treatment was again used as fixed effect. For juvenile BL and WM, treatment included the following five groups: F0 A, F0 Δ1000, F1 C-A, F1 C-Δ1000 and F1 W-Δ1000.

Respirometry

Larval RMR (F1 larvae)

As larvae were reared in a full-factorial design, temperature condition, P_{CO_2} concentration and their interactions were included as fixed effects in the model.

Juvenile SMR and $P_{O_2,crit}$ (F0 and F1 juveniles)

Because of an imbalanced dataset for juvenile respirometry, it was not possible to test the effect of temperature, P_{CO_2} condition, generation, age and their interactions separately; instead, treatment was used as fixed variable, resulting in seven groups for SMR: F0 A-young, F0 Δ1000-young, F0 A-old, F0 Δ1000-old, F1 C-A, F0 C-Δ1000 and F1 W-Δ1000; and five groups for $P_{O_2,crit}$: F0 A-old, F0 Δ1000-old, F1 C-A, F0 C-Δ1000 and F1 W-Δ1000.

RESULTS

Growth

Neither temperature nor P_{CO_2} treatment had a significant effect on larval size at mouth opening stage in F1 larvae (Fig. 2A,D, Table 4). During the following larval development, higher temperatures significantly increased growth if larvae were compared at the same age (dph): F1 C larvae were smaller than F0 and F1 W larvae at higher temperature (Fig. 3A,B, Table 4). SGR ranged from 7.85 to 9.75% day^{-1} for larval DM and 11.67 to 14.76% day^{-1} for larval BL (Table 3). The higher growth rates in F1 W larvae resulted in a Q_{10} of 1.67–2.12 and 1.81–2.35 for DM and BL (Table 3). P_{CO_2} had no effect on the growth of F0 and F1 C larvae, but reduced growth significantly in F1 W larvae (Table 4). Because of the longer larval duration in colder temperatures (900 dd equals 45 dph at 20°C and 60 dph at 15°C), F1 C larvae were of comparable size to F1 W-A and F0 larvae at metamorphosis. In contrast, F1 W-Δ1000 larvae were significantly smaller at metamorphosis than any other group of larvae (Fig. 2B,E, Table 4).

In juveniles, the overall positive effect of temperature on growth persisted, with F1 W juveniles displaying significantly higher growth rates than F1 C juveniles (Fig. 3C,D, Table 4). SGR ranged from 2.88 to 5.16% day^{-1} for juvenile WM and 0.84 to 1.55% day^{-1} for juvenile BL; the higher growth rates resulted in a Q_{10} of 2.41–2.72 and 2.31–2.52 for WM and BL, respectively, in F1 Δ1000 juveniles. If compared at the age of 3000 dd (165, 140 and 181 dph for F0, F1 W and F1 C juveniles, respectively), the difference in size was inverted compared with metamorphosis: F1 W-Δ1000 juveniles were now significantly larger than any other group (Fig. 2C,F, Table 4). P_{CO_2} did not have any significant effect on the growth of F0 or F1 C juveniles. The effect of P_{CO_2} on F1 W juveniles was not determined because of the missing F1 W-A treatment.

Metabolic rate

Metabolic rate estimations were done on larvae with a mean size ranging from approximately 1.5 to 3.0 mg DM and 11.5 to 14 mm BL with no significant differences in size between treatments (BL and DM; Table S3). For juveniles, mean size ranged from approximately 3 to 62 g WM and 9 to 20 cm BL (Table S4), with no significant differences in size (BL and WM) or condition factor between acidification treatments of the same age and generation (ANOVA, $P>0.05$ for F0 old; LME, $P>0.05$ for F0 young and F1 C) or between F1 C and F1 W (LME, $P>0.05$). The positive effect of temperature on growth was mirrored in larval RMR in F1: RMR was significantly lower in F1 C than in F1 W. But in contrast to growth, no effect of

Journal of Experimental Biology (2022) 225, jeb243802. doi:10.1242/jeb.243802

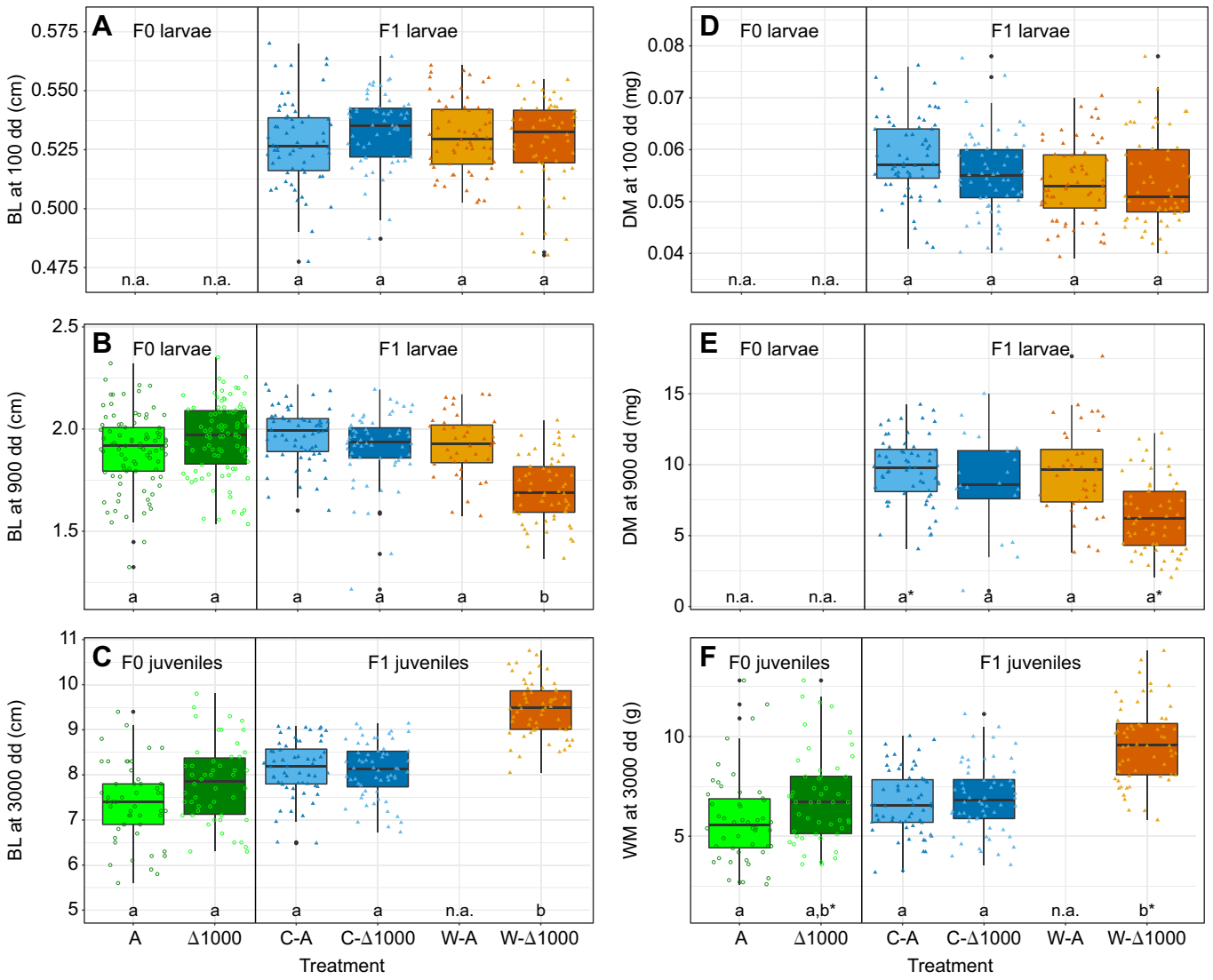

Fig. 2. Body length and mass of European sea bass. Body length (BL) and mass (DM, dry mass; WM, wet mass) data are given for F0 and F1 fish at approximately 100 degree days (dd; mouth opening, 7 dph; A,D), 900 dd (metamorphosis; B,E) and 3000 dd (C,F). Box plots show median, 25th and 75th percentile, 1.5x interquartile range and outliers. Overlying symbols are the individual data points for each treatment; different letters indicate significant differences [linear mixed effect (LME) models, $P<0.05$]; asterisks indicate statistical trends (LME, $P<0.1$). A, ambient P_{CO_2}; Δ1000, ambient+1000 µatm CO_2; C, cold life condition; W, warm life condition; n.a., treatment was not available or not measured in this state. n=40–90.

P_{CO_2} treatment or an interaction of temperature and P_{CO_2} treatment on larval RMR was observed (Fig. 4A, Table 4). A Q_{10} of 2.24 and 2.51 was calculated for larval RMR for F1 A and F1 Δ1000 larvae, respectively. Similarly, juvenile SMR was significantly lower in F1 C than in F1 W juveniles (Fig. 4B, Table 4), with a Q_{10} of 1.61 for F1 Δ1000 juveniles. The comparison between the two generations showed that the SMR in the F0 juveniles did not change significantly between 5 and 15 month old juveniles, but F0 SMR estimates were significantly lower than those in F1 juveniles (Table 4). Comparable to larval RMR, there was no significant effect of P_{CO_2} in juvenile SMR at each thermal treatment. Although the LME model states a significant effect of treatment on $P_{O_2,crit}$ (Fig. 4C, Table 4), *post hoc* tests revealed only a significant difference between F0 Δ1000 and F1 C-A ($P<0.04$); all other groups were not significantly different from each other.

DISCUSSION

Long-term experiments exploring the potential of fish to adapt to OAW are still scarce, especially in larger, temperate species with long generation times. In this long-term experiment, we observed that OW as single driver increased growth rate and RMR in the warm F1 larval sea bass, but as a result of the decreased larval phase duration at warmer temperatures, F1 C-A and F1 W-A larvae had a similar size at metamorphosis. OA as single driver had no effect on F1 larval and juvenile growth or on metabolism at ambient (cold) temperature. Under OAW, F1 W-Δ1000 larvae were significantly smaller at metamorphosis than any other group, while maintaining similar RMR to F1 W-A larvae. As they grew into juveniles, F1 W-Δ1000 fish were bigger than F1 C fish at 3000 dd and had the highest SMR. Unfortunately, the F1 W-A group could not be kept until juvenile phase. Although F0 and F1 W larvae were both raised at increased temperatures, we observed that the detrimental effects of OAW occurred only in F1 W-Δ1000 and not in F0 Δ1000. We also observed that juvenile SMR was lower in F0 than in F1 C and F1 W, with no effect of OA in F0 and F1 C. Juvenile $P_{O_2,crit}$ was not affected by OA or OAW in both generations.

Journal of Experimental Biology (2022) 225, jeb243802. doi:10.1242/jeb.243802

Table 3. Specific growth rate and the respective Q_{10} of F1 larval and juvenile mass and body length of European sea bass

Parameter	Treatment	*n*	0.05 quantile	0.5 quantile	0.95 quantile
Larval dry mass					
SGR (% day^{-1})	C-A	3	9.19±0.37	9.57±0.23	9.63±0.25
	C-Δ1000	1	7.85	9.26	9.75
	W-A	2	12.92±0.38	14.25±0.11	14.76±0.08
	W-Δ1000	3	11.67±0.29	12.92±0.26	13.73±0.20
Q_{10}	A		1.84	1.96	2.12
	Δ1000		1.81	1.67	1.80
Larval body length					
SGR (% day^{-1})	C-A	3	2.27±0.08	2.41±0.04	2.40±0.06
	C-Δ1000	3	2.14±0.07	2.33±0.02	2.43±0.03
	W-A	2	3.09±0.15	3.37±0.06	3.50±0.02
	W-Δ1000	3	2.88±0.01	3.01±0.06	3.26±0.04
Q_{10}	A		1.98	2.22	2.35
	Δ1000		1.81	1.95	1.98
Juvenile wet mass					
SGR (% day^{-1})	C-A	2	3.07±0.09	2.94±0.03	3.04±0.04
	C-Δ1000	2	2.96±0.05	2.88±0.01	2.92±0.04
	W-A		n.a.	n.a.	n.a.
	W-Δ1000	2	5.16±0.02	4.93±0.11	4.82±0.13
Q_{10}	A		n.a.	n.a.	n.a.
	Δ1000		2.72	2.63	2.41
Juvenile body length					
SGR (% day^{-1})	C-A	2	0.91±0.02	0.87±0.00	0.87±0.00
	C-Δ1000	2	0.85±0.02	0.84±0.00	0.86±0.01
	W-A		n.a.	n.a.	n.a.
	W-Δ1000	2	1.55±0.01	1.49±0.02	1.46±0.06
Q_{10}	A		n.a.	n.a.	n.a.
	Δ1000		2.52	2.45	2.31

Specific growth rate (SGR; means±s.e.m. over all replicate tanks per condition) and Q_{10} are given for the 0.05, 0.5 and 0.95 quantile of the cohort. A, ambient P_{CO_2}; Δ1000, ambient+1000 µatm CO_2; C, cold life condition; W, warm life condition; n.a., treatment was not available or not measured at this state.

Effects of OW on European sea bass growth and metabolism

F1 C larvae were reared at 15°C, reflecting ambient temperature towards the middle to the end of the spawning season in the Bay of Brest. We applied a warming scenario of +5°C on F1 W larvae, which reflects typical rearing temperatures in aquaculture, as well as natural temperatures towards the middle to end of the spawning season in the Mediterranean (Ayala et al., 2003). This thermal treatment (20°C) is well below the upper thermal limits for seabass larvae from the Bay of Brest (27°C; Moyano et al., 2017). OW as a single driver at ambient P_{CO_2} significantly increased growth rate and decreased the time to reach metamorphosis in F1 W-A larvae in comparison to F1 C-A larvae. As a result of the longer larval phase duration, size at metamorphosis was comparable between F1 C-A larvae and F1 W-A larvae. Faster growth at higher temperatures and similar size at metamorphosis despite different temperatures has been shown in other studies of sea bass from Mediterranean and Atlantic populations (Ayala et al., 2001, 2003). OW also increased RMR in F1 W-A larvae compared with F1 C-A larvae. The increase in RMR was similar to the increase in SGR, reflected by a similar Q_{10} [1.96, 2.22 and 2.24 for SGR of DM and BL (0.5 quantile), and RMR, respectively]. This reflects the expected Q_{10} increase of 2–3 for biological processes and confirmed our hypothesis that OW will lead to increased growth and RMR in larval sea bass of this particular population. We did not determine the effects of OW as a single driver on growth and metabolism in F1 juveniles because of the absence of F1 W-A.

Effects of OA on European sea bass growth and metabolism

OA as single driver within the cold temperature condition did not affect either growth and metabolism (RMR, SMR) or $P_{O_2,crit}$ in F1 European sea bass larvae or juveniles. In the wild, sea bass eggs are spawned in stable open ocean conditions and larvae develop during drifting towards the coast; therefore, larvae were thought to be less resilient to OA than juveniles and adults. This has already been shown not to be the case for sea bass in scenarios up to SSP5-8.5 and similar (Pope et al., 2014; F0 in Crespel et al., 2017) and was further confirmed by this study, as larval growth and RMR were not affected by OA within the cold temperature group. As juvenile sea bass inhabit coastal areas and have been shown to be tolerant to a broad range of environmental factors, including temperature and salinity (Dalla Via et al., 1998; Claireaux and Lagardère, 1999), their tolerance to OA was expected and could be confirmed in this study – no effects of OA within the cold temperature group on growth, SMR and $P_{O_2,crit}$ were observed. Our study also supports the hypothesis of Montgomery et al. (2019) that an observed 20% decrease in $P_{O_2,crit}$ under an acute increase of P_{CO_2} (3- to 5-fold increase in P_{CO_2} within ∼6 h) in European sea bass will vanish after long-term acclimation to OA.

Combined effects of OA and OW on European sea bass growth and metabolism

However, the combined effects of OA and OW (OAW) changed the picture for larval resilience. While growth rates increased sufficiently in F1 W-A to reach the same size at metamorphosis as F1 C-A, F1 W-Δ1000 larvae were significantly smaller at metamorphosis than larvae from any other treatment, but maintained RMR as high as that of F1 W-A larvae. Q_{10} values revealed that temperature had a stronger effect on metabolic rate than on growth under OA: 1.67 and 1.95 for SGR of DM and BL (0.5 quantile) and 2.51 for RMR, respectively. This suggests that F1 W-Δ1000 larvae either allocated the energy differently, such as using more energy for movement or different regulatory processes,

Journal of Experimental Biology (2022) 225, jeb243802. doi:10.1242/jeb.243802

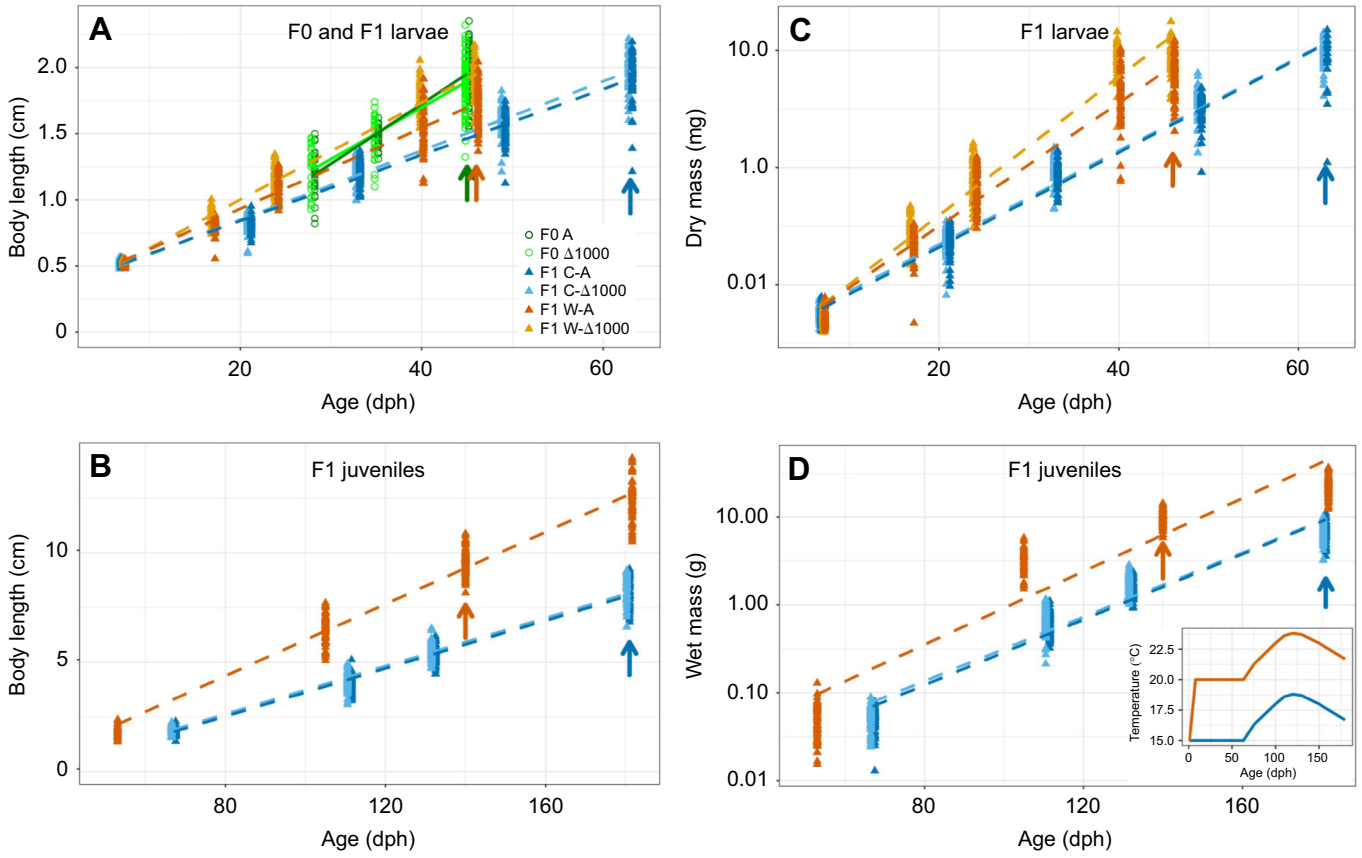

Fig. 3. Growth of F0 and F1 larvae and F1 juveniles with linear regression lines. Shown are individual data points of body length (A,B), larval dry mass (C) and juvenile wet mass (D). F1 C larvae grew significantly slower than F1 W (A,C) and F0 larvae (A). F1 W-Δ1000 larvae grew significantly slower than F1 W-A (A,C) and F0 A larvae (A). F1 W-Δ1000 juveniles grew significantly faster than F1 C juveniles (B,D). No differences were observed between P_{CO_2} treatments in F0 larvae (A), F1 C larvae (A,C) and F1 C juveniles (B,D). All data were tested with LME models; *F*- and *P*-values are summarized in Table 4. Arrows indicate the data points at metamorphosis (900 dd, A,C) and at 3000 dd (B,D); data for different P_{CO_2} conditions of the same age are slightly offset for better visibility. Inset in D shows the temperature history of F1 larvae and juveniles. A, ambient P_{CO_2}; Δ1000, ambient+1000 µatm CO_2; C, cold life condition; W, warm life condition.

or that their energy production and oxygen usage was not as efficient as in the other groups. Although it is possible that the higher RMRs are due to a higher activity of the F1 W-Δ1000 larvae during the measurements, larvae were regularly observed during the trials and the inter-individual variability in movement did not seem related to treatment. Therefore it seems more plausible that larvae under OAW needed energy for different regulatory processes, probably combined with decreased energy production efficiency. In this sense, we previously found that OAW decreased the efficiency of complex II (CII) of the electron transport system (ETS) in cardiac mitochondria of juvenile sea bass in the W-Δ1000 treatment under acute temperature change (Howald et al., 2019). Inhibition of CII by OA was also found in other studies on mammals and fish (Simpson, 1967; Wanders et al., 1983; Strobel et al., 2013). In Atlantic cod embryos, reduced activity of complex I (CI) of the ETS resulted in reduced mitochondrial phosphorylation capacity and subsequently in reduced oxygen consumption rates, while energy requirements were simultaneously increased (Dahlke et al., 2017). Although CII was only affected in juvenile sea bass under acute temperature change, it is probable that larvae are more vulnerable than juveniles (Dahlke et al., 2020a): similar to embryos (Leo et al., 2018), they are less developed while at the same time investing all available energy into growth without reserving excess capacity for environmental regulation and are therefore already affected at their acclimation temperature if OA and OW are combined. This inability to cope with OAW has not been observed in European sea bass larvae before; contrastingly, in former studies, growth of larval European sea bass has been shown to be resilient to OA even at a rearing temperature of 19°C (Pope et al., 2014; F0 larvae in Crespel et al., 2017). Potential explanations why these differences first occurred in F1 are probably related to their parents being reared under OA conditions, as well as effects of different rearing protocols, which are both addressed below (see ‘Effects of OA on European sea bass growth and metabolism over two successive generations’).

In contrast to larvae, F1 W-Δ1000 juveniles displayed a greater thermal plasticity and grew significantly faster than F1 C juveniles, resulting in larger fish at 3000 dd in the F1 W-Δ1000 than in F1 C-A and F1 C-Δ1000 treatments. High growth rates were supported by high SMR, which was also highest in F1 W-Δ1000 juveniles in comparison to F1 C-A and F1 C-Δ1000. As we did not incubate the F1 W-A treatment to juvenile phase, it is unclear whether the detrimental effects of OAW on growth and metabolism in larval European sea bass would have persisted into the juvenile phase. The increased growth rate and bigger size at 3000 dd in F1 W-Δ1000 juveniles in comparison to F1 C-A and F1 C-Δ1000 juveniles might either indicate that OA did not affect growth in juveniles or that growth under OW was so accelerated in juveniles that F1 W-Δ1000 fish were able to catch up and grow to bigger sizes than F1 C fish, masking the negative effects of OAW. The latter suggestion is supported by the findings for SMR and by the Q_{10} of SMR and SGR: in F1 Δ1000 juveniles, SMR was less affected by temperature (Q_{10} 1.61) than SGR [Q_{10} 2.63 and 2.45 for SGR of WM and BL

Journal of Experimental Biology (2022) 225, jeb243802. doi:10.1242/jeb.243802

Table 4. *F*- and *P*-values of fixed effects from the linear mixed effect models on growth and metabolic rate of F0 and F1 larval and juvenile European sea bass

	OAW treatment		P_{CO_2} treatment		Temperature		P_{CO_2}×temperature	
Parameter	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Larval dry mass								
Mouth opening	n.a.	n.a.	1.18	0.3	4.49	0.06	2.13	0.18
Metamorphosis	n.a.	n.a.	11.69	0.01	6.37	0.05	2.73	0.16
Over time	n.a.	n.a.	17.27	0.0032	2.61	0.1447	8.01	0.0221
Larval body length								
Mouth opening	n.a.	n.a.	0.23	0.66	0.21	0.64	1.72	0.23
Metamorphosis	10.04	0.0008	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Over time	275.09	<0.0001	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Juvenile wet mass								
3000 dd	16.41	0.0222	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Over time	240.515	0.0005	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Juvenile body length								
3000 dd	46.93	0.0049	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Over time	1111.59	<0.0001	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
RMR	n.a.	n.a.	0.01	0.94	29.62	<0.0001	0.06	0.82
SMR	95.44	<0.0001	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
$P_{O_2,crit}$	3.79	0.0064	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

OAW, ocean acidification–warming; dd, degree days; RMR, routine metabolic rate; SMR, standard metabolic rate; $P_{O_2,crit}$, critical O_2 concentration; n.a., treatment was not available or not measured in this state.

(0.5 quantile)]. Q_{10} for SGR and SMR is well in the range found in other studies on European sea bass from the Atlantic [Q_{10} for SGR of WM ∼2.4 (15–20°C), calculated from Gourtay et al., 2018; and Q_{10} for SMR 2.09 (14–22°C), calculated from Montgomery et al., 2021 preprint] and from the Western Mediterranean populations [Q_{10} for SGR of WM and RMR of 2.40 and 1.70, respectively (13–25°C), calculated from Person-Le Ruyet et al., 2004]. Person-Le Ruyet et al. (2004) explained the different Q_{10} of RMR and SGR with increased growth rates as being due to increased feed intake. As the fish in our study were fed *ad libitum*, they were able to increase food intake to support high growth rates too. The better capacity of juveniles to cope with and even profit from higher temperatures even under OAW in comparison to larvae is probably a result of the reproduction biology of European sea bass, as well as the generally higher capacity for acid–base regulation in juveniles in comparison to larvae. Larvae develop during spring in the open ocean, where temperatures are stable and relatively cold (8–13°C for Atlantic specimen; Jennings and Pawson, 1992), with optimal larval growth temperatures of 15–17°C (Mediterranean specimens; Koumoundouros et al., 2001; Ayala et al., 2003). In contrast, juveniles live in shallow coastal areas, and so encounter higher temperatures during summer but also higher daily and seasonal variation (6–18°C for Atlantic specimens; Russel et al., 1996) with optimal growth temperatures of 22–28°C (Mediterranean specimens; Lanari et al., 2002; Person-Le Ruyet et al., 2004). Consequently, in terms of growth and metabolism, juvenile sea bass at the northern distribution range might benefit from higher temperatures, as already found in other studies (Howald et al., 2019; Montgomery et al., 2021 preprint), and do not seem to be severely affected by OA.

Effects of OA on European sea bass growth and metabolism over two successive generations

In addition to the effects of the single and combined stressors OA, OW and OAW on individual groups of fish, we also studied the effects of OA in two successive generations on the ability of sea bass larvae and juveniles to cope with upcoming conditions. This study is to our knowledge the first to examine the effects of OA on European sea bass or other long-lived teleosts in more than one generation. Interestingly, the detrimental effect of OAW on larval growth was only observed in F1 and not in F0 larvae of European sea bass, despite their respective parental generation's identical thermal history, and thus appears to be an OA effect. There may be several reasons for this. First, the provisioning of necessary resources when parents have already encountered the same conditions as the future offspring, e.g. via egg size and composition (Munday, 2014), could explain the observed trend in F1 W-Δ1000 larvae. Parental effects can lead in different directions and can last throughout the larval phase: for example, parental effects influence growth in stickleback under OW and OA (Shama et al., 2014; Schade et al., 2014) and explained differences in embryo mortality and hatching success in Atlantic cod under OW (Dahlke et al., 2017). In our study, we did not measure egg size and quality, nor did we incubate offspring of F0 A in cross-factorial Δ1000 scenarios, so we cannot directly quantify parental or transgenerational effects. However, the size of F1 larvae at mouth opening, up to which point the larvae depend on yolk sac reserves, did not differ across treatments. Thus, using this landmark as an indirect indicator, parental provisioning does not seem to explain differences in larval growth rates. Second, the incubation protocol differed between F0 and F1. While F0 larvae were first incubated under OA conditions at 2 dph, F1 sea bass were constantly reared under OA conditions from fertilization onwards, although warming was also applied from 2 dph onwards. It is possible that the effects of OA during embryogenesis shaped the reaction of F1 larvae to OAW, e.g. via epigenetic signalling. As reviewed by Dahlke et al. (2020a), it seems that spawning adults and embryos are the most vulnerable life stages in fish, possessing the lowest tolerance to OW, e.g. Atlantic cod embryos exposed to OAW showed reduced hatching success and oxygen consumption rates (Dahlke et al., 2016) and OA decreased the Q_{10} of RMR in Atlantic silverside embryos (Schwemmer et al., 2020). To summarize, the different reaction of F0 and F1 larvae to OAW could be due to parental effects or effects during embryogenesis and more research is necessary to determine the underlying mechanisms.

As the different temperature life histories and replication schemes (no replicate tanks in F0 juveniles) did not allow a direct comparison of growth rates between F0 and F1 juveniles, we compared size at

Journal of Experimental Biology (2022) 225, jeb243802. doi:10.1242/jeb.243802

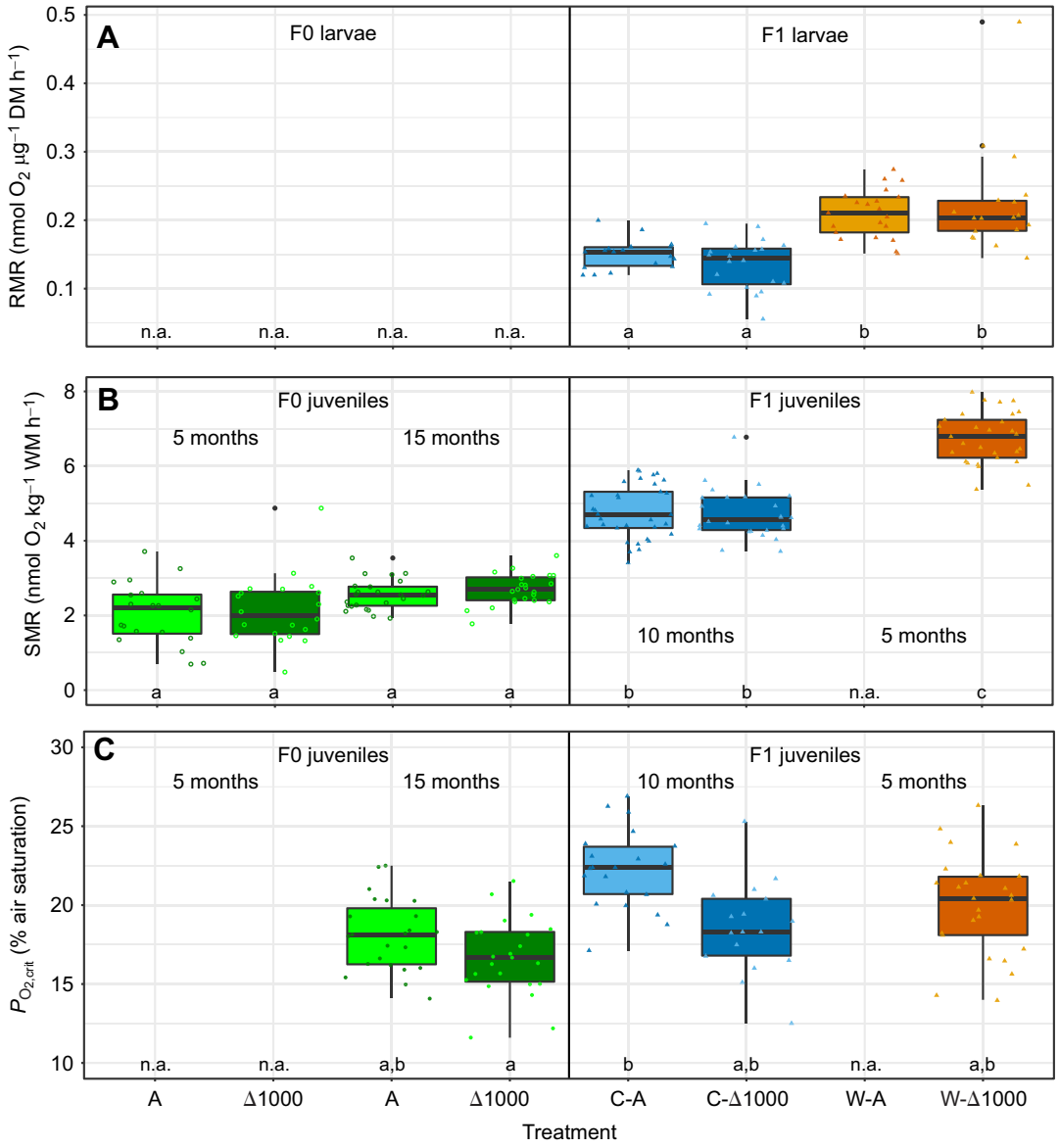

Fig. 4. Routine and standard metabolic rate and critical oxygen concentration of F0 and F1 larvae and juveniles. (A) Routine metabolic rate (RMR), (B) standard metabolic rate (SMR) and (C) critical oxygen concentration ($P_{O_2,crit}$). Box plots show median, 25th and 75th percentile, 1.5x interquartile range and outliers. Overlying symbols are the individual data points for each treatment. Metabolic rates were corrected with allometric scaling factors (0.89 and 0.98 for larvae and juveniles, respectively). Different letters indicate significant differences (LME, P<0.05; data of 15 month old F0 juveniles taken from Crespel et al., 2019). A, ambient P_{CO_2}; Δ1000, ambient+1000 µatm CO_2; C, cold life condition; W, warm life condition; n.a., treatment was not available or not measured in this state. n=20–35.

the age of ∼3000 dd (165, 140 and 181 dph for F0, F1 W and F1 C juveniles). Because of their high growth rates during the juvenile phase, F1 W-Δ1000 fish were largest at 3000 dd, while F0 and F1 C fish were smaller (WM and BL) but similar to each other. This matched the findings for SMR, which was not affected by OA and was higher in F1 W than in F1 C fish. Surprisingly, SMR was also higher in F1 C than in F0 fish. This difference might be explained by the different temperature life histories. While F0 fish had been raised at warmer temperatures and were acclimated to colder temperatures afterwards, F1 C fish had been reared at 15°C throughout their life, except for summer months, when temperatures reached up to 19°C. No detrimental effects on juvenile growth rate under OA were visible in the second generation of sea bass reared under OA conditions, as reflected by similar SMR and SGR between the A and Δ1000 condition. Because of the missing F1 W-A treatment, we cannot state whether the detrimental effects of OAW observed in F1 larvae persisted to the juvenile phase.

Ecological perspective

Larvae are not fully developed compared with later stages and are exposed to higher predation and starvation risks; as such, they had been thought to be more vulnerable to environmental stressors such as OAW (as reviewed in Houde, 2009). In this context, OAW could impact larval survival and recruitment success via different mechanisms. If OAW leads to faster growth rate and increased metabolic rate (as seen in this study between F1 W and F1 C larvae), larvae will need more food in a shorter time to support these growth rates; therefore, it is essential that they match adequate prey fields (prey abundance, size and quality). In our study, the larvae were fed *ad libitum* at both temperatures, supporting increased energetic

demands for the high growth and metabolic rates at higher temperatures. However, in the ocean it is possible that food availability is not sufficient to support accelerated growth under OW. Bochdansky et al. (2005) showed that fish larvae with higher growth and metabolic rates died earlier when food was limited, but profited when fed at saturation level. In sea bass larvae, high growth rates were also only supported under high food ratios, but survival was not significantly decreased, even at one-eighth saturation ratio (Zambonino Infante et al., 1996). This might indicate that sea bass will not grow as fast as in our study under future OW scenarios if food is scarce, but might still survive to juvenile stage.

Besides food-related aspects, OAW can also have a large impact on larval behaviour and dispersal, which can later influence recruitment success. Sea bass spawn in the open ocean and larvae drift inshore (Jennings and Pawson, 1992). As with many temperate species, their swimming behaviour and its effect on dispersal have not been studied as extensively as for coral reef fish that have well-developed sensory abilities (hearing, olfaction, vision) and show directional swimming early on (as reviewed in Leis, 2018; Berenshtein et al., 2021 preprint). To the best of our knowledge, it seems that early sea bass larvae are more dependent on currents than on their swimming performance and that they are able choose a certain depth and therefore a certain current in the preferred direction (Jennings and Pawson, 1992). When drifting closer to the coast, sea bass larvae wait for certain cues from nursery areas, which are present from June onwards (Jennings and Pawson, 1992).

OW accelerates the development of sea bass larvae and therefore possibly alters the timing and spacing of dispersal. Studies have shown species-specific responses of fish behaviour to OA, OW and OAW, e.g. OW increased activity level in larval kingfish but not boldness, while OA had no effect on these behavioural traits (Laubenstein et al., 2019). Yet, OA decreased swimming duration and orientation in larval dolphinfish (Pimentel et al., 2014) and reversed orientation towards settlement habitat cues in barramundi (Rossi et al., 2015). To our knowledge, larval sea bass behaviour has not been measured under OAW yet. Consequently, because of the altered timing of larval development and in combination with the possibility of altered behaviour and impacted senses, reaching nursery areas might be challenging for sea bass larvae under OAW, especially if (1) food is not abundant and (2) cues are weaker and/or different as a result of the greater distance and/or earlier timing. Once the larvae enter the coastal areas and metamorphose, they are exposed to a more changing environment. Although this study confirmed that juvenile sea bass are less vulnerable to OAW than larval sea bass, food availability and behaviour will determine whether the observed increased growth under OAW in F1 will occur in the wild too. In a sister study on offspring of wild-caught European sea bass, OAW reduced digestive enzyme activity under restricted food ratios, resulting in severely reduced food conversion efficiency and reduced growth rates (Cominassi et al., 2020). Additionally, OA decreased the distance over which early juvenile sea bass sensed food or predator cues (Porteus et al., 2018) and juvenile sea bass behaviour was altered by OW, resulting in a decreased latency of the escape response and mirror responsiveness (Manciocco et al., 2015). Consequently, although faster larval (OW) and juvenile growth (OAW) as well as earlier metamorphosis (OW, OAW) are generally beneficial for larvae and early juveniles, many factors may modulate this effect and whether it will translate into higher larval survival, recruitment and increased growth rates in the wild. Further research should determine the effects of limited food under OAW on larval and juvenile growth and behaviour.

As the hypoxia tolerance of European sea bass juveniles was unaffected by OA, OW and OAW, they might cope well with upcoming hypoxia events in coastal areas. However, it is important to note here that we measured $P_{O_2,crit}$ only at SMR and thus may have estimated P_{O_2} effects too conservatively. Recent studies suggest that this $P_{O_2,crit}$ at SMR might not be the most ecologically relevant estimate (see Seibel and Deutsch, 2020, and references therein). Long-term survival of individuals and the population would require that the fish are able to digest food, grow and reproduce, which would require more energy than provided by SMR. Consequently, depending on the duration and intensity of hypoxia events, individuals might be able to survive in the short term, but other fitness-related traits such as growth might be affected in the long term.

Conclusion

We confirmed our hypotheses that OW increases growth and metabolism in the European sea bass, and that larvae as well as juveniles are resilient to OA if it occurs as a single stressor. We also confirmed that OAW has detrimental effects on larval growth. Our results, together with other findings on larval fish and European sea bass suggest that it is possible that under OAW, fewer individuals will reach metamorphosis, e.g. as a result of limited food to support high growth rates, different dispersal to nursery areas by altered developmental timing, changed behaviour or altered olfactory senses. However, those individuals that reach the juvenile phase might benefit from higher temperatures, because of increased performance.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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

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Data availability

Datasets of growth, metabolic rates and water conditions during rearing are available from PANGAEA: https://doi.pangaea.de/10.1594/PANGAEA.941767

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