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Lack of detrimental effects of ocean acidification and warming on proximate composition, fitness and energy budget of juvenile Senegalese sole (*Solea senegalensis*)



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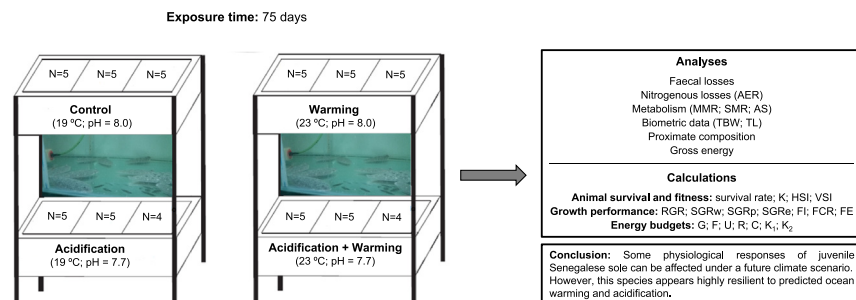
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HIGHLIGHTS

- Climate change can affect nutritional quality and physiology of marine organisms.
- Growth, metabolism and excretion were assessed under acidification and warming.
- Weight gain, metabolic rates and energy intake increased under future climate conditions.
- The highest energy budget fractions were allocated to growth and faecal excretion.
- Juvenile Senegalese sole is resilient to climate change-related scenarios.

GRAPHICAL ABSTRACT



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ABSTRACT

Rising levels of atmospheric carbon dioxide (CO₂) are driving ocean warming and acidification, which may negatively affect the nutritional quality and physiological performance of commercially important fish species. Thus, this study aimed to evaluate the effects of ocean acidification (OA; ΔpH = -0.3 units equivalent to ΔpCO₂ ~ +600 μatm) and warming (OW; ΔT = +4 °C) (and combined, OAW) on the proximate composition, fitness and energy budget of juvenile Senegalese sole (*Solea senegalensis*). After an exposure period of 75 days, growth (G), metabolism (R) and excretion (faecal, F and nitrogenous losses, U) were assessed to calculate the energy intake (C). Biometric and viscera weight data were also registered to determine animal fitness. Overall, the proximate composition and gross energy were not significantly affected by acidification and warming (alone or in combination). Weight gain, maximum and standard metabolic rates (MMR and SMR, respectively), aerobic scope (AS) and C were significantly higher in fish subjected to OA, OW and OAW than in CTR conditions. Furthermore, the highest relative growth rates (RGR), specific growth rates in terms of wet weight (SGRw) and protein (SGRp), as well as feed efficiencies (FE) occurred in fish

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submitted to OW and OAW. On the other hand, fish exposed to CTR conditions had significantly higher feed conversion ratio (FCR) and ammonia excretion rate (AER) than those exposed to simulated stressors. Regarding energy distribution, the highest fraction was generally allocated to growth (48–63 %), followed by excretion through faeces (36–51 %), respiration (approximately 1 %) and ammonia excretion (0.1–0.2 %) in all treatments. Therefore, ocean warming and acidification can trigger physiological responses in juvenile Senegalese sole, particularly in their energy budget, which can affect the energy flow and allocation of its population. However, and in general, this species seems highly resilient to these predicted ocean climate change stressors.

1. Introduction

Climate change has been one of the most severe threats to the stability of global marine ecosystems, being mainly attributed to drastic anthropogenic perturbations. Ocean acidification (OA) and warming (OW) are among the most significant chronic environmental issues linked to climate change. The high temperature in conjunction with reduced seawater pH levels has been instigated by high atmospheric carbon dioxide (CO₂) concentrations (that are sequestered into ocean surface waters) (IPCC, 2021, 2022). Currently, atmospheric CO₂ concentration has exceeded 400 ppm and is predicted to reach >1000 ppm by the end of the 21st century (IPCC, 2021; NOAA, 2022). Additionally, the Intergovernmental Panel on Climate Change (IPCC) also predicted that ocean pH will further reduce by 0.3–0.4 units (i.e., from pH 8.1 to pH 7.7) and the seawater surface temperature will rise between 1 and 4 °C by the end of this century (due to the release of exorbitant greenhouse gas emissions). In the coming decades, OA and OW are expected to occur concurrently at an unprecedented rate (RCP 8.5 scenario), hence it is essential to consider both stressors when forecasting responses to climate change (IPCC, 2021).

It has been reported that environmental disturbances resulting from global climate change can elicit negative impacts on marine life and ecosystem functioning. Therefore, the rapidly changing process has drawn substantial concerns to the fitness and survival of marine organisms, many of them being expected to diminish or even disappear in the near future (Poloczanska et al., 2016; IPCC, 2022). According to Poloczanska et al. (2016), OA and OW can affect almost all aspects of marine organism physiology.

The effect of increasing water temperature on the proximate composition and physiological responses (e.g., growth performance, feed conversion ratio, metabolism [i.e., oxygen consumption] and energy budget) of several fish species has been already studied in the past (e.g., Guinea and Fernandez, 1997; Requena et al., 1997; Kausar and Salim, 2006; Fang et al., 2010; Anacleto et al., 2018). Guerreiro et al. (2012) also evaluated the effect of temperature (16 and 22 °C) and dietary protein/lipid ratio on growth performance, proximate composition and nutrient utilization (feed efficiency) of juvenile Senegalese sole. Alongside, the impacts of projected OA on fish proximate composition, survival, growth (including in different early life stages, such as larval), metabolic rate and energy allocation have also been assessed in previous studies (e.g., Munday et al., 2009; Kim et al., 2013; Shuangyao et al., 2018; Machado et al., 2020; Tegomo et al., 2021). Moreover, the influence of near-future OW and OA (acting in combination) on the nutritional quality and physiological functioning (e.g., survival, growth - in different early life stages -, assimilation efficiency, metabolism, ammonia excretion rate (AER), cellular energy allocation) of some commercially important marine species like turbinid snails, sea urchins, rainbow trout, pacific herring and yellowfin bream has also been addressed (Morgan et al., 2001; Ab Lah et al., 2018; Coleman et al., 2019; Villalobos et al., 2020; Harianto et al., 2021; Zhang et al., 2022). The majority of these studies suggest that under the projected scenarios of OA and OW (acting alone or in combination) by 2100 and beyond, significant changes on nutritional quality and physiological performance (e.g., growth, metabolism, excretion) are expected, with some marine species appearing more vulnerable and others more resilient (e.g., Anacleto et al., 2018; Ab Lah et al., 2018; Coleman et al., 2019; Camacho et al., 2020; Shalders et al., 2022). Therefore, it is also clear from the literature

that the effect of multi-stressor environments and their interactions is variable and undoubtedly species-specific. For instance, Shalders et al. (2022) reported that OA and OW can lead to reductions in seafood essential nutrients such as protein and lipids, while Coleman et al. (2019) did not observed changes in proximate composition of yellowfin bream (*Acanthopagrus australis*). On the other hand, Anacleto et al. (2018) observed an increase of the protein and ash contents in juvenile white seabream (*Diplodus sargus*) under warming conditions. It was also mentioned that the species that seem to be more resilient in terms of nutritional quality are those that inhabit naturally variable environments such as shallow near-coastal habitats and estuaries (Shalders et al., 2022). Yet, to the best of our knowledge, there are no studies evaluating the interactive effects of OA and OW on the energy budget of benthic and temperate fish species (such as Senegalese sole). In this context, understanding the effects of these stressors (combined or acting alone) is a key priority to assess the fitness, response, and population dynamics of marine organisms under the forecasted environmental changes, which will be crucial for the sustainable management of future fisheries and aquaculture. Furthermore, understanding the consequences of climate change on the oceans is urgently needed to rapidly identify and implement possible mitigation and adaptation measures (Poloczanska et al., 2016; Weatherdon et al., 2016). Hence, the aim of this work was to evaluate the effect of ocean acidification (i.e., $\Delta\text{pH} = -0.3$ units equivalent to $\Delta\text{pCO}_2 \sim +600 \mu\text{atm}$) and warming (i.e., $\Delta\text{T} = +4$ °C) (acting alone or in combination) expected for 2100 (IPCC, 2021, 2022) on the proximate composition, fitness and energy budget of juvenile Senegalese sole after 75 days of exposure. This benthic and temperate flatfish with poor swimming capabilities was selected as biological model due to its specific ecological characteristics and high commercial and economic importance to both fisheries and aquaculture sectors (e.g., farming production of *S. senegalensis* and *Solea solea* in Portugal increased from 83 tons in 2014 to 160 tons in 2020) (Camacho et al., 2020; Machado et al., 2020; FEAP, 2021). This species is a marine ectotherm, i.e., body functioning largely depending on external environmental factors, such as temperature, making it vulnerable to hydrographic variations in the area where inhabits (Pimentel et al., 2014; Baag and Mandal, 2022).

2. Materials and methods

2.1. Experimental design and sampling

Juvenile Senegalese sole (*S. senegalensis*: 25.2 ± 5.1 g total weight; 12.7 ± 1.3 cm total length; mean \pm standard deviation) were reared at the Aquaculture Research Station of the Portuguese Institute for the Sea and Atmosphere (EPPA-IPMA, Olhão, Portugal) and transported under controlled conditions (temperature, dissolved oxygen) to the Guia Marine Laboratory (MARE-FCUL, Cascais, Portugal). Upon arrival, fish were randomly distributed (initial density ± 5 g body weight L⁻¹) into 12 rectangular-shaped holding tanks (length \times width \times height = $98 \times 33 \times 25$ cm; 80 L volume). Each holding tank ($n = 12$) was connected to independent recirculating aquaculture systems (RAS, $n = 12$) equipped with physical (protein skimmer; Reef SkimPro, TMC Iberia, Portugal) and biological filtration (FSBF 1500, TMC Iberia, Portugal), besides UV disinfection (Vecton 300, TMC Iberia, Portugal). Within each RAS, temperature was controlled through seawater chilling systems (Frimar, Fernando Ribeiro Lda, Portugal) and pH by means of an automatic control device (Profilux 3.1N,

GHL, Germany) linked to a solenoid valve system. Independent pH measurement and regulation of gases injection were performed by using pH electrodes ($n = 12$) connected to the Profilux system. pH monitoring was performed every 2 s and upon demand, values were lowered by injection of a certified CO_2 gas mixture (Air Liquide, Portugal) or increased by tank aeration (via air stones) with CO_2 -filtered air (using soda lime, Sigma-Aldrich, USA). Seawater was continuously UV sterilized in the RAS before being supplied to the tanks (Vecton 600, TMC Iberia, Portugal). On a daily basis, total ammonia ($\text{NH}_3/\text{NH}_4^+$), nitrite (NO_2^-) and nitrate (NO_3^-) were monitored by means of colorimetric tests (Tropic Marin, USA) and levels were maintained below 0.05 mg L^{-1} , 0.20 mg L^{-1} and 2.0 mg L^{-1} , respectively; mortality was registered, and filtered seawater ($0.35 \mu\text{m}$) was partially replaced (approximately 20 %) in the systems to maintain an optimum quality. Total alkalinity (TA) was determined in each tank on a weekly basis according to the method described by Sarazin et al. (1999) and the carbonate system parameters were calculated through the combination of TA and pH values [see Supplementary (Supp.) Table S1 for more details].

Fish were acclimated to laboratory conditions during 30 days under the following abiotic conditions: dissolved oxygen above 7 mg L^{-1} ; temperature = $19.0 \pm 0.4 \text{ }^\circ\text{C}$; pH = 8.06 ± 0.10 ; salinity = $35.5 \pm 0.5 \text{ }‰$ (WTW handheld Meter Multi 350i, Germany); and photoperiod of 12 h/12 h light/dark cycle. To minimize stress and allow acclimation to experimental conditions, before initiating exposure treatments, seawater temperature was slowly increased ($1 \text{ }^\circ\text{C}$ per day) and pH slowly lowered (-0.1 pH units per day) until reaching $23 \text{ }^\circ\text{C}$ and/or $\sim 1000 \mu\text{atm}$ $p\text{CO}_2$ (equivalent to pH = 7.7 units). Fish were exposed to four experimental treatments for a time period of 75 days: i) Control (CTR) - temperature = $19 \text{ }^\circ\text{C}$ and pH = 8.0 ($p\text{CO}_2 \sim 400 \mu\text{atm}$) (current conditions in Iberian Peninsula Senegalese sole farms; Pousão-Ferreira, 2009); ii) Ocean Acidification (OA) - temperature = $19 \text{ }^\circ\text{C}$ and pH = 7.7 ($p\text{CO}_2 \sim 1000 \mu\text{atm}$); iii) Ocean Warming (OW) - temperature = $23 \text{ }^\circ\text{C}$ and pH = 8.0 ($p\text{CO}_2 \sim 400 \mu\text{atm}$); and iv) Ocean Acidification and Warming (OAW) - temperature = $23 \text{ }^\circ\text{C}$ and pH = 7.7 ($p\text{CO}_2 \sim 1000 \mu\text{atm}$) (Supp. Table S1). Each treatment comprised three independent replicate tanks ($n = 4\text{--}5$ individuals per tank, i.e., 14–15 fish per treatment). During the experimental trials, fish were daily fed (2 % of their average body weight, divided by two meals) with a fish-based diet manufactured by SPAROS, Lda (Olhão, Portugal) (detailed formulation and proximate composition can be found in Supp. Table S2), following the nutritional requirements of this species. The feeding protocol (i.e., amount of feed) was weekly adjusted, according to the growth and appetite of the fish.

At the end of the trial (T75), specimens were randomly sampled from each tank ($n = 8$ per treatment for determination of energy budget parameters, except in the case of metabolism, where an $n = 4$ was tested per treatment). The nitrogenous losses (non-faecal excretion) and oxygen consumption rates - OCR (metabolic rates) were assessed in live fish, as explained in Subsections 2.3.2 and 2.4, respectively. Subsequently, individuals were euthanized by immersion in 2000 mg L^{-1} of tricaine methanesulfonate (MS-222; Sigma-Aldrich, USA) buffered with sodium bicarbonate (NaHCO_3 ; Sigma-Aldrich, USA) using a ratio of 1:1 for 10 min. Biometric data (total body weight - TBW; total length - TL) were recorded, and the euthanized fish were dissected to weight the liver and other viscera in order to assess animal and organ condition (see Section 2.5.1). Faeces were collected through the intestine dissection method (Peres et al., 2013) to determine faecal losses (see Section 2.3.1). Those specimens were then homogenised (Retsch Grindomix GM200, Haan, Germany; 5000 rpm), freeze-dried for 48 h at $-50 \text{ }^\circ\text{C}$ under low pressure (approximately 10^{-1} atm ; Power Dry 150 LL3000, Heto, Czech Republic) and stored at $-80 \text{ }^\circ\text{C}$ until determination of gross energy content. Three fish were also randomly collected from each treatment and homogenised as described above to assess the proximate composition (see Section 2.2). Fish trials were approved by the Ethical Committee of the Faculty of Sciences of the Lisbon University (ORBEA) and conducted according to legal EU regulations (EU Directive 2010/63).

2.2. Proximate composition and gross energy

Moisture, ash, free fat, crude protein (%) and gross energy ($\text{kJ } 100 \text{ g}^{-1}$) were determined at the beginning and end of the experiment (T0 and T75, respectively) in the fish according to the Association of Official Analytical Chemists methods (AOAC, 2005). Briefly, moisture was determined by oven drying (lab heater, P-Selecta 207, Barcelona, Spain) the sample overnight at $105 \pm 1 \text{ }^\circ\text{C}$, whereas ash was obtained by incineration of dried sample in a muffle furnace (TYP.MR170, Heraeus, Hanau, Germany) for 16 h at $500 \pm 25 \text{ }^\circ\text{C}$. Free fat was determined through the Soxhlet extraction method (in a Soxhlet apparatus, Behr Labor-Technik, Dusseldorf, Germany) using diethyl ether solvent (at approximately $40 \text{ }^\circ\text{C}$; 7 h), and by weighing the fat residue after drying ($105 \pm 1 \text{ }^\circ\text{C}$) in an oven. Crude protein was calculated from total nitrogen using the conversion factor of 6.25 (FAO, 2003). Total nitrogen was analysed according to the Dumas method (Saint-Denis and Goupy, 2004) in an automatic nitrogen analyser (LECO FP-528, LECO Corp., St. Joseph, USA) calibrated with EDTA. Nitrogen was released by combustion at $850 \text{ }^\circ\text{C}$ and detected by thermal conductivity. Gross energy was determined by combustion in an adiabatic bomb calorimeter (Werke C2000, IKA, Staufen, Germany) calibrated with benzoic acid.

2.3. Excretion

2.3.1. Faecal losses

The faeces of Senegalese sole were obtained through the intestine dissection method due to the difficulty of collecting them, since they are semi-liquid and thus dissolve very fast in water, as reported by other authors (e.g., Peres et al., 2013). The collection was performed approximately 3 h after the last meal at days 25, 50 and 75. Faeces of individuals from the same tank were pooled whenever necessary to assure a good representativeness of samples; they were then oven-dried (overnight) at $65 \text{ }^\circ\text{C}$, weighted, homogenised, stored at $-20 \text{ }^\circ\text{C}$ and analysed in terms of energy using the same technique described in Section 2.2.

2.3.2. Nitrogenous losses (non-faecal excretion)

Excreted nitrogenous compounds (ammonia-N) were assessed at the end of the trial (T75) using individual live fish enclosed in 2 L glass bottles for 1 h for each treatment. Controls without fish (three blanks per tank) were also performed and ammonia levels were measured to evaluate the potential loss of nitrogenous compounds through bacterial action or diffusion in the experimental tanks. The bottles were filled with oxygen saturated seawater and sealed without any air bubbles. Trials were done in the same conditions (temperature, pH and salinity) of the corresponding treatment. The seawater was filtered ($0.45 \mu\text{m}$) and frozen in 20 mL plastic flasks for quantification of ammonia, which was determined according to Berthelot method (Grasshoff, 1983). Samples were treated with alkaline citrate, sodium hypochloride and phenol in the presence of sodium nitroprussiate which catalyses the reaction. The blue colour formed by indophenol plus ammonia reaction was measured at 630 nm. Ammonia excretion rate (AER; expressed as $\text{mg g}^{-1} \text{ ww L}^{-1} \text{ h}^{-1}$) was calculated as follows:

$$\text{AER} = \frac{\Delta[\text{NH}_4^+]}{\text{VH}_2\text{O}/\text{TBW}/\Delta T}$$

where:

- $\Delta[\text{NH}_4^+]$ is the difference between the ammonia levels in the sample and blank (mg);
- VH_2O is the volume of water (L) in the bottle;
- TBW is the fish total wet weight (g);
- ΔT is the time duration of the trial (hours).

2.4. Metabolism

At the end of the experiment (T75), respiration trials were conducted in an intermittent flow-through respirometer system to measure oxygen

consumption rates and to determine the maximum metabolic rate (MMR) and standard metabolic rate (SMR). To measure MMR, an exhaustive chase protocol was followed (Clark et al., 2013; Rummer et al., 2016), which consisted in placing the fish in a bucket and chasing them manually for 3 min, until exhaustion, i.e., until they did not respond to further stimulation. Subsequently, fish were exposed to air for 60 s and quickly placed in a respirometer chamber (volume: 1.2 L, Loligo Systems, Tjele, Denmark) where oxygen consumption was recorded in each minute over the first 10 min of recovery using the Pyro Oxygen Logger software (PyroScience, Germany). The SMR was determined according to previously established methods (Anacleto et al., 2018). Metabolic data were analysed after importing the text file from the FireSting O₂ software (FireSting O₂, PyroScience, GmbH, Aachen, Germany) into Excel. Linear regressions between seawater oxygen concentration and time were made for each measurement period and the slopes derived from the regressions were used to calculate metabolic rates - MR (MMR and SMR, mg O₂ kg⁻¹ ww h⁻¹) according to the following equation:

$$MR = \frac{b \times V \times t}{W}$$

where:

b is the slope of the oxygen reduction curve (minus the blank control);
V is the volume of the respirometer minus the volume of the fish assuming that 1 kg of seawater is equivalent to 1 kg of fish (in L);
t is the time interval over which O₂ is assessed (in hours);
W is the wet weight of fish (in kg).

The aerobic scope (AS) was calculated as the difference between SMR and MMR (Clark et al., 2013).

Moreover, O:N ratios were calculated by dividing the amount (atoms) of oxygen consumed by the nitrogen excreted, being used to estimate what substrates (protein, lipid and carbohydrate) the organisms use for metabolism (Babarro et al., 2000):

$$O : N = \frac{SMR/16}{AER/17}$$

2.5. Data calculation

2.5.1. Animal survival and fitness

The survival rate (%) of fish was calculated as a percent of the final number per initial number in each tank. The Fulton's condition index (K) was directly calculated from the biometric data to determine fish condition, as the total weight (g) divided by total length (cm) cubed $\times 100$ (Ricker, 1975). The organ weight (g) as a percent of the total fish weight (g) was calculated for the liver (hepatosomatic index, HSI, %) and the viscera (i.e., all other organs except liver; viscerosomatic index, VSI, %), in order to provide information on liver and viscera condition.

2.5.2. Growth performance

Relative growth rate (RGR, %), specific growth rate in terms of wet weight, protein and energy (SGRw, SGRp and SGRe, respectively, % day⁻¹), feed intake (FI, g g⁻¹ body weight day⁻¹), feed conversion ratio (FCR) and feed efficiency (FE) were calculated according to the following equations (Fang et al., 2010; Shuangyao et al., 2018):

$$RGR = \frac{(W2 - W1)}{W1} \times 100$$

$$SGRw = \frac{(\ln W2 - \ln W1)}{t} \times 100$$

$$SGRp = \frac{(\ln P2 - \ln P1)}{t} \times 100$$

$$SGRe = \frac{(\ln E2 - \ln E1)}{t} \times 100$$

$$FI = \frac{F}{(t \times (W2 + W1)/2)} \times 100$$

$$FCR = \frac{FI}{(W2 - W1)}$$

$$FE = \frac{(W2 - W1)}{F}$$

where:

W2 and W1 are the final and initial body weights of the fish (g), respectively;

P2 and P1 are the final and initial protein contents (g 100 g⁻¹), respectively;

E2 and E1 are the final and initial energy contents (J g⁻¹), respectively;
t is the duration of the experiment (days);

F is the dry weight of feed intake during the experimental period (g).

2.5.3. Energy budgets

Energy budgets (J g⁻¹ day⁻¹) were calculated according to the model proposed by Carfoot (1987):

$$C = G + F + U + R$$

where:

C is the energy consumed;

G is the energy accumulated for growth;

F is the energy lost in faeces;

U is the energy lost through ammonia excretion;

R is the energy lost for respiration.

The G value was calculated as follows:

$$G = (W2 \times E2) - (W1 \times E1)$$

where:

W2 and W1 are the final and initial wet body weights of the fish (g), respectively;

E2 and E1 are the final and initial energy contents of the fish (J g⁻¹), respectively.

The F values were determined as referred in Section 2.3.1. For determination of U values, the levels of ammonia-N (AER) were multiplied by the coefficient of 24.83 kJ g⁻¹ N and, consequently, converted into energy (Elliott, 1976). The R values were obtained through the conversion of the oxygen consumption into energy using a factor of 13.84 J mg⁻¹ O₂ (442.88 J mmol⁻¹ O₂) (Guinea and Fernandez, 1997).

Finally, the assimilation efficiency (K₁, %) and net growth efficiency (K₂, %) were calculated using the following equations (Ye et al., 2009):

$$K_1 = \frac{(G + R)}{(G + R + U)} \times 100$$

$$K_2 = \frac{G}{(G + R)} \times 100$$

2.6. Statistical analysis

Statistical analysis was performed using the STATISTICA software version 12 (StatSoft Inc., Tulsa, OK, USA). All data were checked for normality of distribution and homogeneity of variances using Kolmogorov-Smirnov and Levene's tests, respectively. The effect of pH

and temperature on analysed endpoints was tested by factorial analysis of variance (ANOVA), with replicate tank as random effect (see results in Supp. Tables S3, S4, S5, S6 and S7). Tukey's HSD test was applied in groups multiple comparisons. Whenever ANOVA assumptions were not met even after data transformation, the non-parametric Kruskal-Wallis rank sum test followed by multiple comparisons was applied to detect significant differences between treatments (see Supp. Table S8). Statistical significance was considered at $p < 0.05$ for all analyses (Zar, 2010).

3. Results and discussion

3.1. Animal survival and fitness

The different pH and temperature conditions tested did not affect the survival rate of juvenile Senegalese sole, which remained at 100 % throughout the 75-day trial. Therefore, this species can withstand the tested seawater acidification and/or warming scenarios under controlled conditions. Moreover, the indexes calculated to assess fish, liver and viscera condition (i.e., K, HSI and VSI; mean values between 1.2 and 1.5, close to 1 % and 3 %, respectively, Table 1) were not significantly affected by pH and temperature variations ($p > 0.05$). Similar results were reported earlier for: i) juvenile Senegalese sole exposed to warming ($\Delta T = +4$ °C; temperature set at 19 and 23 °C) and/or acidification ($\Delta pH = -0.4$ units; pH set at 8.0 and 7.6 units) for 28 days (only for K; Camacho et al., 2020); ii) juvenile white seabream submitted to warming ($\Delta T = +5$ °C; temperature set at 19 and 24 °C) for 56 days (only for HSI; Anacleto et al., 2018); and iii) black seabream (*Acanthopagrus schlegelii*) exposed to acidification ($\Delta pH = -0.3$ and -0.7 units; pH set at 8.1, 7.8 and 7.4 units) for 8 weeks (only for HSI; Tegomo et al., 2021). The absence of significant differences between treatments in K and VSI indicates similar fish and viscera conditions, while for HSI it shows similar feeding intensities (Ricker, 1975; Tegomo et al., 2021). Thus, an environmental change in seawater pH and temperature does not seem to have direct consequences to the fitness of juvenile Senegalese sole, indicating that this may be a resilient species to such a change.

Table 1

Biometry, fitness data and feed intake, conversion ratio and efficiency of juvenile Senegalese sole (*Solea senegalensis*) at day 0 and after 75 days of exposure to pH and temperature experimental conditions.

Parameter	CTR	OA	OW	OAW
Day 0				
TBW (g)	24.06 ± 5.91	28.30 ± 5.16	23.38 ± 4.61	25.78 ± 4.40
TL (cm)	12.21 ± 1.24 ^b	13.93 ± 1.35 ^a	12.24 ± 0.84 ^b	12.97 ± 0.98 ^{a,b}
K	1.31 ± 0.31	1.11 ± 0.13	1.28 ± 0.21	1.22 ± 0.27
Day 75				
TBW (g)	60.81 ± 10.77 ^b	82.44 ± 7.00 ^a	81.17 ± 12.41 ^a	80.93 ± 6.79 ^a
ΔW (g)	36.99 ± 8.62 ^b	52.92 ± 4.95 ^a	53.50 ± 6.04 ^a	57.16 ± 6.56 ^a
TL (cm)	17.00 ± 1.12	18.18 ± 1.31	18.01 ± 1.16	18.50 ± 0.87
K	1.24 ± 0.22	1.23 ± 0.17	1.45 ± 0.14	1.33 ± 0.17
HSI (%)	1.15 ± 0.28	1.05 ± 0.31	0.91 ± 0.14	0.88 ± 0.17
VSI (%)	3.34 ± 0.81	2.74 ± 0.63	2.68 ± 0.64	2.59 ± 0.57
FI	2.29 ± 0.26 ^a	2.12 ± 0.12 ^{a,b}	1.96 ± 0.24 ^{a,b}	1.85 ± 0.13 ^b
(g g ⁻¹ day ⁻¹)				
FCR	0.07 ± 0.02 ^a	0.04 ± 0.00 ^b	0.04 ± 0.01 ^b	0.03 ± 0.01 ^b
FE	0.50 ± 0.11 ^b	0.60 ± 0.03 ^b	0.79 ± 0.05 ^a	0.79 ± 0.09 ^a

Results are given as mean values ± standard deviation ($n = 14-15$ [T0] or $n = 8$ [T75]). For each parameter, different superscript letters (a–b) in the same row indicate significant differences ($p < 0.05$) between treatments. CTR (Control): $T = 19$ °C; pH 8.0 ($pCO_2 \sim 400$ μatm); OA (Acidification): $T = 19$ °C; pH 7.7 ($pCO_2 \sim 1000$ μatm); OW (Warming): $T = 23$ °C; pH 8.0 ($pCO_2 \sim 400$ μatm); OAW (Acidification + Warming): $T = 23$ °C; pH 7.7 ($pCO_2 \sim 1000$ μatm). TBW: Total body weight; TL: Total length; K: Fulton's condition index; ΔW : Weight variation = [TBW(T75)-TBW(T0)]; HSI: Hepatosomatic index; VSI: Viscerosomatic index; FI: Feed intake; FCR: Feed conversion ratio; FE: Feed efficiency.

3.2. Proximate composition and gross energy

The proximate composition and gross energy obtained in juvenile Senegalese sole exposed to different climate change-related scenarios are summarised in Table 2. Moisture, protein, fat and ash contents were not significantly affected by acidification and warming conditions (acting alone or in combination) ($p > 0.05$). The gross energy was significantly higher in OA (548 ± 13 kJ 100 g⁻¹) than in CTR (T75: 515 ± 11 kJ 100 g⁻¹) and OW (509 ± 29 kJ 100 g⁻¹), likely due to intrinsic variability between individuals (Tukey's HSD test, $p = 0.007$ and $p = 0.002$, respectively). The outcomes of proximate composition are in accordance with those reported for cod (*Gadus morhua*) (in larval phase) during 46 days post-hatch (Frommel et al., 2011), ornamental shrimp (*Neocaridina heteropoda*) over a 90-day trial period (Tropea et al., 2015), turbinid snail (*Turbo militaris*) exposed for 38 days (Ab Lah et al., 2018) and black seabream (*Acanthopagrus schlegelii*) for 8 weeks of exposure (Tegomo et al., 2021) in trials simulating warming and/or acidification. Anacleto et al. (2018) also observed that the gross energy in juvenile white seabream was not affected after 56 days of exposure to warming. Hence, *S. senegalensis* seems to maintain the nutritional quality (in terms of proximate composition) under acidification and/or warming conditions during 75 days.

3.3. Growth performance

Morphometric data (TBW and TL) of juvenile Senegalese sole registered for each treatment in the two sampling periods (days 0 and 75) are presented in Table 1. After 75 days of exposure, the values of both parameters increased in all treatments, as expected. In the case of weight, the mean gain observed in fish subjected to OA, OW and OAW ($\Delta W = \sim 55$ g) was significantly higher than that obtained in fish exposed to CTR treatment ($\Delta W = 37$ g) (two-way ANOVA, $F \geq 5.1$, $p \leq 0.035$, Table S3; Tukey's HSD test, $p \leq 0.008$). Furthermore, statistically higher RGRs, SGRW (s) and SGRp(s) were observed in fish exposed to warming acting in combination with acidification (OAW; mean values: 241 %, 1.6 % day⁻¹ and 0.07 % day⁻¹, respectively) and in isolation (OW; mean values: 215 %, 1.7 % day⁻¹ and 0.06 % day⁻¹, respectively) compared to the CTR (mean values: 157 %, 1.3 % day⁻¹, 0.02 % day⁻¹, respectively) (Tukey's HSD test, $p \leq 0.032$ and $p \leq 0.035$, respectively) (Fig. 1A, B and D). The RGR and SGRp found in OAW were also significantly higher than those observed in OA (mean values: 180 % and 0.04 % day⁻¹, respectively) ($p = 0.008$ and $p = 0.031$, respectively). It should be noted that the SGR_e and SGR_p values (Fig. 1C and D) were very low in all treatments (mean values: ≤ 0.05 % day⁻¹ and ≤ 0.07 % day⁻¹, respectively). Thus, the growth (in terms of wet weight, RGR, SGR_w and SGR_p) of Senegalese sole was affected by seawater warming and acidification (combined or

Table 2

Proximate composition and gross energy of juvenile Senegalese sole (*Solea senegalensis*) at day 0 and after 75 days of exposure to pH and temperature experimental conditions.

Parameter	Day 0		Day 75			
	CTR		CTR	OA	OW	OAW
Moisture (%)	75.7 ± 2.1		75.5 ± 0.6	74.5 ± 1.0	75.5 ± 0.8	74.1 ± 1.6
Protein (%)	15.4 ± 1.1		15.7 ± 0.2	16.6 ± 0.7	16.2 ± 0.5	17.3 ± 0.9
Fat (%)	3.8 ± 0.2		4.1 ± 0.3 ^{a,b}	4.7 ± 0.3 ^a	3.8 ± 0.1 ^b	4.2 ± 0.3 ^{a,b}
Ash (%)	3.4 ± 0.7		2.7 ± 0.3	3.3 ± 0.3	2.8 ± 0.1	3.3 ± 0.6
Gross energy (kJ 100 g ⁻¹)	503 ± 25		515 ± 11 ^b	548 ± 13 ^a	509 ± 29 ^b	529 ± 15 ^{a,b}

Results are given as mean values ± standard deviation ($n = 3$ for proximate composition and $n = 8$ for gross energy). For each parameter, different superscript letters (a–b) indicate significant differences ($p < 0.05$) between the four treatments (T75). CTR (Control): $T = 19$ °C; pH 8.0 ($pCO_2 \sim 400$ μatm); OA (Acidification): $T = 19$ °C; pH 7.7 ($pCO_2 \sim 1000$ μatm); OW (Warming): $T = 23$ °C; pH 8.0 ($pCO_2 \sim 400$ μatm); OAW (Acidification + Warming): $T = 23$ °C; pH 7.7 ($pCO_2 \sim 1000$ μatm).

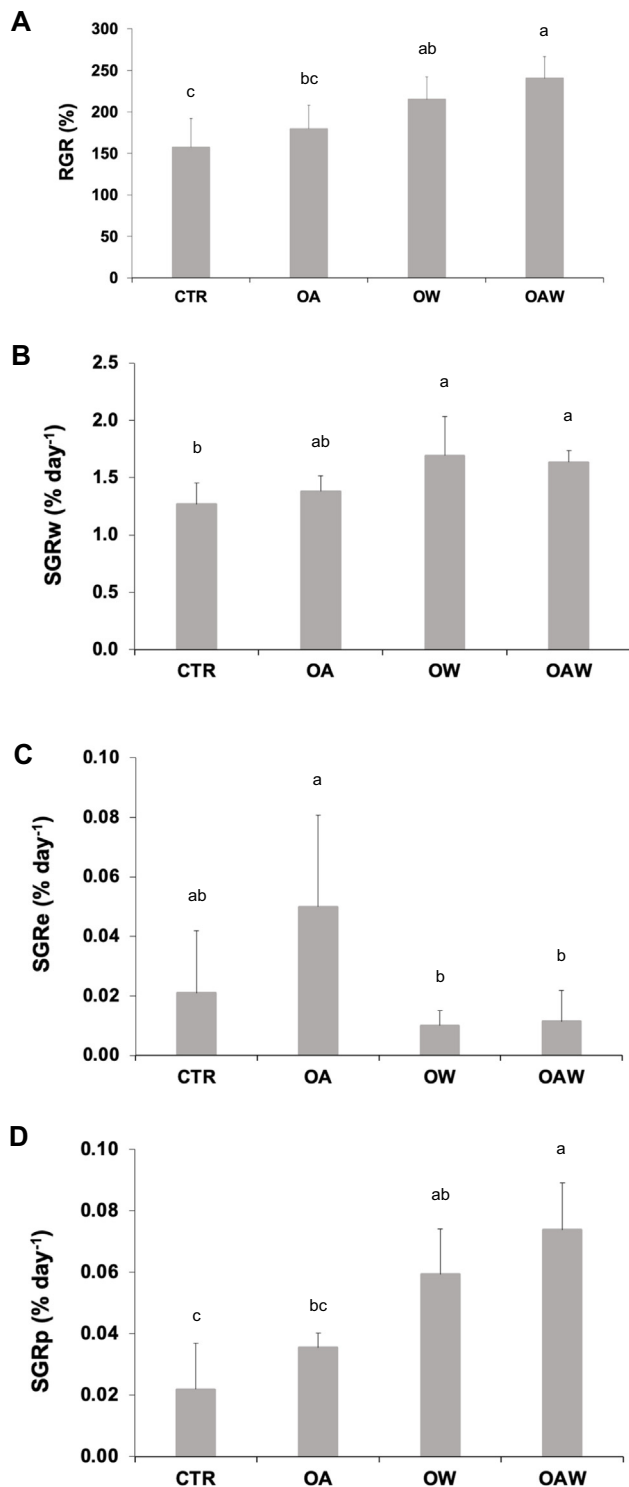


Fig. 1. A) Relative growth rate (RGR) and B, C, D) specific growth rates in terms of wet weight (SGRw), energy (SGRe) and protein (SGRp), respectively in juvenile Senegalese sole (*Solea senegalensis*) after 75 days of exposure to pH and temperature experimental conditions. Results corresponds to mean values \pm standard deviation ($n = 8$ for RGR, SGRw and SGRe; $n = 3$ for SGRp). Different letters (a–c) indicate significant differences ($p < 0.05$) between treatments. CTR (Control): $T = 19$ °C; pH 8.0 ($p\text{CO}_2 \sim 400$ μatm); OA (Acidification): $T = 19$ °C; pH 7.7 ($p\text{CO}_2 \sim 1000$ μatm); OW (Warming): $T = 23$ °C; pH 8.0 ($p\text{CO}_2 \sim 400$ μatm); OAW (Acidification + Warming): $T = 23$ °C; pH 7.7 ($p\text{CO}_2 \sim 1000$ μatm).

not). Previous studies also indicated that the growth of fish (e.g., marine, temperate, tropical, benthopelagic) increased with temperature (Kausar and Salim, 2006; Anacleto et al., 2018; Coleman et al., 2019) and with

CO_2 concentration (i.e., decreasing pH) (Morgan et al., 2001; Munday et al., 2009; Kim et al., 2013; Shuangyao et al., 2018). This result has been attributed to the fact that higher temperatures increase fish metabolic rates, and consequently, the nutritional requirements and feed intake (Kausar and Salim, 2006; Anacleto et al., 2018). Nonetheless, in the present study, it should be noted that fish exposed to warming (acting alone or in combination with acidification) did not present higher FI levels compared to those submitted to CTR conditions (Table 1). Kausar and Salim (2006) also reported that an increase in temperature increases the activity of digestive enzymes, which may accelerate the digestion of nutrients, resulting in higher growth. On the other hand, enhanced growth performance in acidified water has been related to increased energy intake or reduced energy expenditure (Kim et al., 2013; Shuangyao et al., 2018). For instance, Morgan et al. (2001) observed that acid-exposed rainbow trout exhibited increased appetite to compensate for greater branchial ion loss (to maintain their osmotic balance) and beyond that the associated increased energy intake enhanced the growth rate. However, the TL found in Senegalese sole was not affected by seawater pH and temperature variations at the end of the trial (Table 1, $p > 0.05$), as also observed by Camacho et al. (2020).

FI was significantly lower in OAW compared to CTR (highest level) (Tukey's HSD test, $p = 0.004$), likely due to fish weight variations observed in both treatments (Table 1). Thus, since FI did not vary much between the four treatments and the weight variation was significantly higher in fish subjected to acidification and warming (acting alone or in combination), the FCR were significantly lower in the three simulated climate change treatments (0.03–0.04) compared to the CTR (0.07) (Tukey's HSD test, $p \leq 0.010$) (Table 1). Other authors also reported that warming and acidification (acting alone) influenced fish FCR since lower values were found at higher water temperatures and lower pH (Kausar and Salim, 2006; Shuangyao et al., 2018, respectively). Additionally, fish exposed to OW and OAW revealed significantly better FE mean values (0.79) than those subjected to CTR (0.50) and OA (0.60) (Tukey's HSD test, $p \leq 0.007$) treatments (Table 1). A similar effect of warming (acting alone) on FE was reported by Guerreiro et al. (2012) and Anacleto et al. (2018). A potential explanation for improved FE in fish maintained at higher temperature can be the less energy required for the thermoregulation process (Kausar and Salim, 2006).

3.4. Nitrogenous losses and metabolism

Generally, MMR, SMR, AS, as well as AER were significantly affected by warming and acidification (acting alone or in combination) (Fig. 2A and B). Fish exposed to OA, OW and OAW had significantly lower AER compared to those exposed to CTR conditions (Tukey's HSD test, $p = 0.038$, $p \leq 0.001$ and $p = 0.037$, respectively). This probable adaptive response to the simulated conditions can be explained by a decrease in protein catabolism in the intestinal lumen during digestion or a reduction in cellular amino acid oxidation, which may have resulted in a lower ammonium excretion from tissues (Buckling, 2017). Furthermore, Ip and Chew (2010) also mentioned that under adverse environmental conditions, where ammonia excretion is reduced, some fish can decrease its production rate from amino acid catabolism to slow down the build-up of ammonia internally. However, all AER mean values were very low (≤ 0.020 $\text{mg g}^{-1} \text{L}^{-1} \text{h}^{-1}$) compared to those found in a previous study performed with juvenile white seabream (app. 0.15 $\text{mg g}^{-1} \text{L}^{-1} \text{h}^{-1}$), where the temperature tested ($\Delta T = +5$ °C; 19 and 24 °C) (acting alone) had no significant effect on AER (Anacleto et al., 2018). Additionally, Harianto et al. (2021) found that, in some cases, sea urchins (*Heliocidaris erythrogramma*) acclimated to elevated temperatures (+2 °C and +3 °C, corresponding to a sea surface temperature of approximately 24.5 and 25.5 °C) had significantly higher AERs, with no effect under decreased pH conditions (from 8.0 to 7.6), as well as no interaction between temperature and pH. Therefore, contradictory AER results under climate change stressors were reported for different seafood species probably due to extrinsic (e.g., salinity, pH, temperature) but also intrinsic (e.g., life history and developmental impacts) factors (Buckling, 2017).

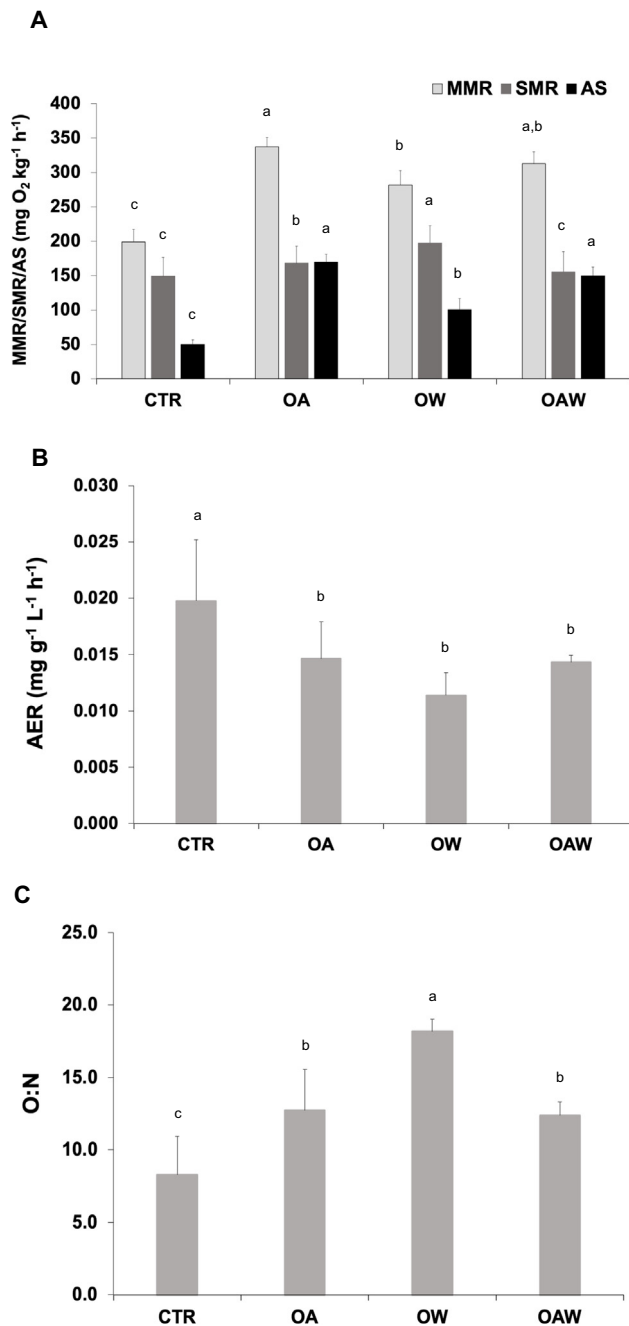


Fig. 2. A) Maximum metabolic rate (MMR), standard metabolic rate (SMR), aerobic scope (AS), B) ammonia excretion rate (AER) and C) O:N ratio of juvenile Senegalese sole (*Solea senegalensis*) after 75 days of exposure to pH and temperature experimental conditions. Results corresponds to mean values \pm standard deviation ($n = 4$ for MMR, SMR, AS and O:N; $n = 8$ for AER). Different letters (a–c) indicate significant differences ($p < 0.05$) between treatments. CTR (Control): $T = 19^\circ\text{C}$; pH 8.0 ($p\text{CO}_2 \sim 400 \mu\text{atm}$); OA (Acidification): $T = 19^\circ\text{C}$; pH 7.7 ($p\text{CO}_2 \sim 1000 \mu\text{atm}$); OW (Warming): $T = 23^\circ\text{C}$; pH 8.0 ($p\text{CO}_2 \sim 400 \mu\text{atm}$); OAW (Acidification + Warming): $T = 23^\circ\text{C}$; pH 7.7 ($p\text{CO}_2 \sim 1000 \mu\text{atm}$).

In contrast to the results observed in AER, MMR and AS values were significantly higher in fish subjected to OA, OW and OAW compared to those exposed to CTR conditions (Tukey's HSD test, $p \leq 0.002$ and $p \leq 0.004$, respectively). The same pattern was observed for SMR (except in the case of OAW) (Tukey's HSD test, $p \leq 0.00001$). In addition, the highest MMR and AS values were found for OA (337.4 ± 13.6 and $169.6 \pm 11.5 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$, respectively) and OAW (312.6 ± 17.3 and $149.5 \pm 12.9 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$, respectively). It is also noteworthy that MMR level

obtained under OA was significantly higher than that found in OW ($281.6 \pm 21.1 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) ($p = 0.021$). Similarly, AS values under OA and OAW were significantly higher than that observed in OW ($100.5 \pm 16.0 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) ($p = 0.001$ and $p = 0.005$, respectively). These findings suggest an increase or maintenance of aerobic performance under future levels of ocean acidification and warming (acting alone or in combination) for Senegalese sole. An increase of OCR in fish species exposed to warming conditions was also reported in previous studies, indicating that a higher temperature causes a general stimulation of metabolism, i.e., in oxygen consumption (Requena et al., 1997; Morgan et al., 2001; Pimentel et al., 2014; Anacleto et al., 2018). A recent study also reported similar SMR results in juvenile Senegalese sole exposed to acidification conditions. However, the authors found that the MMR and AS were not significantly affected by the pH range similar to that tested in this study (i.e., between pH = 8.1 and pH = 7.6) (Machado et al., 2020). On the other hand, Hannan and Rummer (2018) mentioned that an increase in AS was observed in fish species upon $p\text{CO}_2$ exposure to the range encompassing 900 to 2000 μatm , i.e., a range covering the CO_2 partial pressure tested in the present study - 1000 μatm . According to these authors the tolerance or insensitivity to elevated $p\text{CO}_2$ can be explained at the mechanistic level, where the haemoglobins that are highly pH sensitive (in fishes) coupled with plasma-accessible carbonic anhydrase can enhance oxygen delivery to select tissues throughout a mild acidosis (Hannan and Rummer, 2018). Thus, juvenile Senegalese sole seems to be tolerant to the higher temperature (23°C) and lower pH (7.7) tested in terms of metabolic performance.

The O:N ratio was significantly lower in fish subjected to CTR conditions (8.3 ± 2.7) than in those exposed to climate change treatments (Tukey's HSD test, $p \leq 0.002$). Moreover, the ratio obtained under OW (18.2 ± 0.9) was also significantly higher than those found in OA and OAW (12.7 ± 2.8 and 12.4 ± 0.9 , respectively) ($p = 0.0002$) (Fig. 2C). These values reflected fish physiological status in terms of oxygen consumption (SMR) and ammonia excretion (AER), already discussed above. Furthermore, such results were lower than 30, indicating a prevalence of protein-dominated catabolism (i.e., conditions of alimentary deficiency) (Macé and Ansell, 1982) in all scenarios. Similar O:N ratios (<30) were obtained by Anacleto et al. (2018) for juvenile white seabream exposed to warming conditions.

3.5. Energy budgets

Energy spent in different physiological processes and energy intake from feed can be found in Table 3. The energy expended for growth (G) and energy acquired from feed (C) were significantly higher in fish exposed to OA, OW and OAW (3727 ± 141 to $4099 \pm 182 \text{ J g}^{-1} \text{ day}^{-1}$ and 6227 ± 293 to $6490 \pm 263 \text{ J g}^{-1} \text{ day}^{-1}$, respectively) than in those subjected to CTR conditions ($2416 \pm 486 \text{ J g}^{-1} \text{ day}^{-1}$ and $4918 \pm 418 \text{ J g}^{-1} \text{ day}^{-1}$, respectively) (Tukey's HSD test, $p = 0.0002$). Additionally, pH and temperature variations (i.e., -0.3 units and $+4^\circ\text{C}$, respectively) did not influence energy lost through faeces (F) ($p > 0.05$) that ranged from 2317 ± 220 to $2455 \pm 173 \text{ J g}^{-1} \text{ day}^{-1}$. On the other hand, the energy lost via ammonia excretion (U) was significantly higher in fish exposed to CTR conditions ($11.8 \pm 3.2 \text{ J g}^{-1} \text{ day}^{-1}$) than in those maintained under climate change conditions (6.8 ± 1.2 to $8.7 \pm 2.0 \text{ J g}^{-1} \text{ day}^{-1}$) (Tukey's HSD test, $p = 0.038$, $p = 0.0002$ and $p = 0.037$ when comparing with OA, OW and OAW, respectively). Finally, fish exposed to OW significantly used more energy for respiration (R) ($64.5 \pm 2.6 \text{ J g}^{-1} \text{ day}^{-1}$) than those submitted to CTR ($49.4 \pm 6.1 \text{ J g}^{-1} \text{ day}^{-1}$) and OAW ($54.9 \pm 2.2 \text{ J g}^{-1} \text{ day}^{-1}$) conditions (Tukey's HSD test, $p = 0.004$ and $p = 0.043$, respectively).

Regarding energy distribution, the highest mean fraction was allocated to growth (60–63%), followed by excretion through faeces (36–39%), respiration (app. 1%) and ammonia excretion (0.1%) under the three climate change scenarios. In the case of CTR treatment, a similar mean fraction was allocated to excretion via faeces (51%) and growth (48%), followed by respiration (1%) and ammonia excretion (0.2%). Therefore, energy allocated

Table 3

Energy budget and allocation, assimilation and net growth efficiency of juvenile Senegalese sole (*Solea senegalensis*) after 75 days of exposure to pH and temperature experimental conditions.

Parameter	CTR	OA	OW	OAW
Energy budget (J g ⁻¹ day ⁻¹ ww)				
G	2416 ± 486 ^b	3727 ± 141 ^a	3810 ± 292 ^a	4099 ± 182 ^a
F	2455 ± 173	2425 ± 284	2362 ± 184	2317 ± 220
U	11.8 ± 3.2 ^a	8.7 ± 2.0 ^b	6.8 ± 1.2 ^b	8.5 ± 0.4 ^b
R	49.4 ± 6.1 ^b	55.7 ± 0.7 ^{ab}	64.5 ± 2.6 ^a	54.9 ± 2.2 ^b
C	4918 ± 418 ^b	6227 ± 293 ^a	6260 ± 302 ^a	6490 ± 263 ^a
Energy allocation (%)				
G	48.3 ± 3.0 ^b	60.1 ± 2.5 ^a	61.6 ± 1.4 ^a	63.2 ± 1.8 ^a
F	50.5 ± 2.9 ^a	38.8 ± 2.5 ^b	37.2 ± 1.4 ^b	35.8 ± 1.8 ^b
U	0.24 ± 0.08 ^a	0.14 ± 0.03 ^b	0.11 ± 0.02 ^b	0.13 ± 0.01 ^b
R	1.02 ± 0.10 ^a	0.90 ± 0.05 ^b	1.03 ± 0.05 ^a	0.85 ± 0.10 ^b
Efficiency coefficients (%)				
K ₁	99.4 ± 0.1 ^b	99.8 ± 0.1 ^a	99.8 ± 0.0 ^a	99.8 ± 0.0 ^a
K ₂	98.0 ± 0.2 ^c	98.5 ± 0.1 ^{ab}	98.3 ± 0.1 ^{b,c}	98.6 ± 0.1 ^a

Results are given as mean values ± standard deviation (n = 8). For each parameter, different superscript letters (a–c) indicate significant differences (p < 0.05) between treatments. CTR (Control): T = 19 °C; pH 7.7 (pCO₂ ~ 1000 µatm); OA (Acidification): T = 19 °C; pH 7.7 (pCO₂ ~ 1000 µatm); OW (Warming): T = 23 °C; pH 8.0 (pCO₂ ~ 400 µatm); OAW (Acidification + Warming): T = 23 °C; pH 7.7 (pCO₂ ~ 1000 µatm); G: energy loss for growth; F: energy loss through faeces; U: energy loss through ammonia excretion; R: energy loss for respiration; C: energy intake (C = G + R + F + U); K₁: assimilation efficiency; K₂: net growth efficiency.

to growth and lost via faeces significantly increased and decreased, respectively under climate change conditions (Tukey's HSD test, p = 0.0002) (Table 3). Additionally, in the four scenarios, the assimilation efficiency (K₁) and net growth efficiency (K₂) ranged between 99.4 ± 0.1 and 99.8 ± 0.1 % and 98.0 ± 0.2 to 98.6 ± 0.1 %, respectively, being the highest values found in fish subjected to OA, OW and OAW. Such values were significantly different from those observed in CTR conditions (with the exception of that found in OW for K₂) (Kruskal-Wallis test; multiple comparisons, CTR vs OA: p = 0.047 (K₁) and p = 0.001 (K₂); CTR vs OW: p = 0.0001 (K₁) and p = 0.851 (K₂); and CTR vs OAW: p = 0.023 (K₁) and p = 0.0001 (K₂)) (Table 3). Similar K₁, but lower K₂ (approximately 100 % and 70 %, respectively) were reported for juvenile white seabream after 56 days of exposure to warming conditions. However, in this study, temperature did not significantly affect these two parameters (Anacleto et al., 2018).

Overall, the obtained outcomes indicate that exposure to a lower pH and/or higher temperature affects energy budget parameters, as well as the assimilation and net growth efficiencies, as observed in previous studies for other seafood species (Anacleto et al., 2018; Shuangyao et al., 2018; Zhang et al., 2022). In terms of energy distribution, similar and contradictory results were found in literature. For instance, Anacleto et al. (2018) also reported that the higher and lower proportion of food energy in juvenile white seabream was allocated to growth (56.0–67.8 %) and ammonia losses (2.3–3.3 %), respectively after 56 days of exposure to warming conditions (ΔT = +5 °C). In contrast, Shuangyao et al. (2018) concluded that most of the food energy in juvenile turbot (*Scophthalmus maximus*) was used in metabolism (app. 72 %), followed by growth (app. 27 %), faeces loss and nitrogenous excretion after 8 weeks of exposure to acidification conditions (pH from 6.3 ± 0.2 to 8.8 ± 0.2). Recently, Zhang et al. (2022) observed that the physiological adjustments and energy strategies of sea urchins (*Strongylocentrotus intermedius* and *Tripneustes gratilla*), a temperate and a tropical species, respectively) towards exposure to seawater acidification (pH = 7.6) and warming (ambient temperature + 3 °C, i.e., 24 °C and 30 °C, respectively) for 28 days were species-specific. These authors also mentioned that the changing environmental factors can strongly affect the energy balance in marine organisms due to the increased energy consumption for physiological adjustments (Zhang et al., 2022). Besides

temperature and pH, it is necessary to take into account that other factors (e.g., body size, life cycle, feed type or composition, difficulty to find and consume food, and environment-specific stressors, such as predator density) can also influence the energy budget of an organism (Anacleto et al., 2018).

4. Conclusion

The obtained results clearly show that i) the juvenile Senegalese sole is resilient under seawater acidification and/or warming conditions (e.g., the survival rate remained at 100 % throughout the 75-day experiment); ii) changes in seawater pH and/or temperature do not seem to have direct consequences on proximate composition, gross energy, TL, animal fitness (K, HSI and VSI) and energy lost through faeces; iii) acidification and warming (acting alone or in combination) can promote higher growth (in terms of weight, RGR, SGRw, SGRp, being more evident for OW and OAW), FE, MMR, SMR, AS and energy intake (C), as well as lower FCR and AER. Additionally, in the present study, the energy proportion spent for growth dominated the mode of energy allocation of juvenile Senegalese sole and was followed by excretion through faeces under the three climate change scenarios (OA, OW and OAW). In the case of fish exposed to CTR conditions, a similar fraction was allocated to excretion via faeces and growth. Thus, some physiological responses can be affected in this species under a realistic future climate scenario. Future research should address the effect of climate change stressors (acidification, warming and others such as hypoxia) on prey distribution and availability, in populations of organisms that interact with Senegalese sole, as well as on another commercially valuable seafood species to better forecast the biological responses in our oceans.

CRedit authorship contribution statement

Helena Oliveira: Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Ana Luísa Maulvault:** Methodology, Investigation, Writing – review & editing. **Sara Castanho:** Methodology, Investigation, Writing – review & editing. **Tiago Repolho:** Methodology, Investigation, Writing – review & editing. **Luísa M.P. Valente:** Methodology, Investigation. **Pedro Pousão-Ferreira:** Investigation, Resources. **Rui Rosa:** Conceptualization, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **António Marques:** Conceptualization, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Patrícia Anacleto:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.159491>.

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