fishes

MDPI

Article

Lactiplantibacillus plantarum I Induces Gonad Growth in the Queen Scallop *Aequipecten opercularis* (Linnaeus, 1758) under Conditions of Climate Change

Ines Kovačić [1], Petra Burić [2,*], Iva Čanak [3], Ante Žunec [2], Anamarija Panić [2], Valentina Kolić [2], Neven Iveša [2], Jadranka Frece [3] and Mauro Štifanić [2]

[1] Faculty of Educational Sciences, Juraj Dobrila University of Pula, Zagrebačka 30, 52100 Pula, Croatia; ikovacic@unipu.hr
[2] Faculty of Natural Sciences, Juraj Dobrila University of Pula, Zagrebačka 30, 52100 Pula, Croatia; azunec@unipu.hr (A.Ž.); anamarijapanic03@gmail.com (A.P.); valy.kolic@gmail.com (V.K.); nivesa@unipu.hr (N.I.); mstifanic@unipu.hr (M.Š.)
[3] Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia; icanak@pbf.hr (I.Č.); jfrece@pbf.hr (J.F.)
* Correspondence: petra.buric@unipu.hr

Abstract: Climate change has presented a serious problem in recent times, which is why a new approach is being sought in terms of aquacultural food quality. In this study, the influence of temperature increase (by 2 °C) and pH decrease (by 0.2) was investigated on the queen scallop, *Aequipecten opercularis* (Linnaeus, 1758). Furthermore, the effect of a food-enriched diet with the probiotic culture *Lactiplantibacillus plantarum* I was assessed in climate-changed conditions. Scallops' morphometric parameters were measured before the experimental setup and after one month of being kept in controlled conditions. Morphometric parameters included the elongation index, compactness index, convexity index, density index, condition index, meat yield, gonadosomatic index, adductor muscle index, and hepatosomatic index. Climate-changed conditions had no effect on the scallop condition index, meat yield, or hepatosomatic index. Nevertheless, the addition of probiotics to their diet had a positive effect on the queen scallops cultivated under conditions of climate change, influencing positive allometry and the increase of the gonadosomatic indices. On the other hand, the same conditions negatively affected the adductor muscle index of the scallops. To conclude, in the context of climate change conditions, queen scallops could be a good organism of choice that can be very well adapted to the changed environmental conditions, especially with the addition of the lactic acid bacteria culture *Lpb. plantarum* I.

Keywords: queen scallop; climate change; mariculture; morphometric indices; probiotic culture

Key Contribution: The impact of the probiotic *Lactiplantibacillus plantarum* I had a positive effect on queen scallop *Aequipecten opercularis* cultivated under conditions of climate change, influencing positive allometry and the increase of the condition and gonadosomatic indices.

check for updates

Citation: Kovačić, I.; Burić, P.; Čanak, I.; Žunec, A.; Panić, A.; Kolić, V.; Iveša, N.; Frece, J.; Štifanić, M.. *Lactiplantibacillus plantarum* I Induces Gonad Growth in the Queen Scallop *Aequipecten opercularis* (Linnaeus, 1758) under Conditions of Climate Change. *Fishes* **2024**, *9*, 326. https://doi.org/10.3390/fishes9080326

Academic Editor: Rodolphe Elie Gozlan

Received: 25 April 2024
Revised: 26 July 2024
Accepted: 17 August 2024
Published: 19 August 2024

1. Introduction

Climate change today represents one of the biggest challenges that humanity has to face in order to protect the environment and the lives of all species on Earth. All climate models indicate a trend of temperature increase as a consequence of the increased level of greenhouse gases [1–3]. Climate change is already having a major impact on the marine ecosystem, mostly due to the increase in sea temperature and the decrease in the pH value of seawater. Predictions point to the fact that the sea temperature could rise by 2 °C over the next 50 years [1]. Since the start of the Industrial Revolution, the global mean sea surface pH has dropped by 0.1 units (from 8.2 to 8.1), equivalent to a 30% increase in ocean acidity.

Anthropogenic climate change is expected to cause a further decrease in global mean sea surface pH by 0.32 units, which could reach pH 7.4 by 2300 [2].

The sea temperature increase for the Mediterranean basin was recorded to be 0.4 °C between 1989–2009 [4]. The Adriatic Sea is one of the 12 units that the Mediterranean basin is divided into and is therefore considered a sea sensitive to climate change. Research in the Adriatic Sea has demonstrated decadal fluctuations in sea surface temperature [5–7]. Namely, the average monthly sea surface temperatures revealed a cooling trend before 1979 and a significant warming after 2008 [5]. Furthermore, the mean temperature rise rate from 1946 to 2015 was noted to be 1.3 ± 0.5 °C per century [8]. In another study, it was stated that temperature, together with salinity and macronutrient availability, will be the main drivers of changes in the area of the Po' river delta, which is the Northern Adriatic Sea [9]. Therefore, due to the vast effect of ocean warming on marine populations and fisheries over the past 15 years [10,11], it is important to explore growth patterns, particularly in relation to parameters such as sea temperature and pH [12]. In one study, 55 of the 90 Mediterranean threatened species were characterized by high vulnerability to climate change. In the aforementioned study, the species that were recorded to have high vulnerability regarding climate change, especially the elevation of the temperature, were noted to be Anthozoa (n = 13 of 16), Chondrichthyes (n = 30 of 45), Actinopterygii (n = 6 of 18), marine mammals (n = 2 of 6), sea turtles (n = 2 of 3), Bivalvia (n = 1), and Malacostraca (n = 1) [13].

The swift warming of coastal and estuarine habitats has prompted pressing inquiries regarding the future of shellfish fisheries and aquaculture [14]. Human nutrition in the Mediterranean region is largely based on the intake of food of marine origin, mostly different types of fish, crustaceans, and shellfish. In the age of global warming, the flourishing of mariculture, the increase in intensity, and the economic benefit that comes with it created problems of reduced water and soil quality and disease outbreaks due to bacterial infections of cultivated organisms [15]. The increase in seawater temperature is causing higher mortality rates in farmed shellfish cultures due to frequent diseases caused by the rapid growth of microbial pathogens. The main consequence is the loss of an entire population of organisms, which leads not only to economic problems in the industry but also to irregular supply of the targeted market [16]. Almost all edible shellfish fall into the categories of oysters, mussels, and scallops, which are used in environmental quality monitoring programs [17–20]. Depending on the type of shellfish, the range of tolerance for changes in temperature and pH values differs, and once it crosses this limit, mortality rates increase rapidly [21]. Scientific research points to the growing global trend of mass mortality of farmed mussels, clams, oysters, and scallops, affecting all life stages of shellfish, from larvae to young and adult individuals [2,22]. Sea acidification makes it difficult for many organisms to survive, especially bivalves. The disadvantage of bivalves can be seen in the fact that at a reduced pH, bivalves invest less energy to create shells so that the internal soft tissue continues to develop. Acidification also reduces the availability of carbonate minerals that bivalves use to build their shells, leading to abnormal and reduced shell growth. In these conditions, bivalves are more susceptible to shell destruction in nature [23]. In addition to acidification, the rising sea temperatures serve as a catalyst for the proliferation and reproduction of marine organisms, including pathogenic species. This environmental shift enhances the potential for infections in other marine organisms. Furthermore, the increase in average air temperatures will significantly increase the risk of shellfish spoilage during storage and distribution to consumers. Additionally, it is also necessary to consider the negative impact of temperature increases on the bioenergetics of bivalves, which leads to excessive energy consumption, affecting their growth, reproduction, and immunity, which can lead to premature death of the organism [2]. For this reason, it is necessary to find an alternative species of shellfish well adapted to a wider range of environmental conditions, especially to the expected increase in temperature and decrease in pH value.

In our previous research, we have shown that an adequate choice of an alternative bivalve species for such cultivation is the queen scallop, *Aequipecten opercularis* (Linnaeus,

1758) [20,24,25]. Scallops have been farmed commercially in Europe for more than 100 years, and directed fisheries in most countries began in the 1930s. The annual catch of *A. opercularis* varied between 7000 and 16,000 tons for a long period, with overfishing occurring in 1995 and 1996. An increase in the annual catch to 30,000 tons was recorded in 2013, which represented 31% of the total catch of scallops [26]. There is no controlled breeding of this species in Croatia, but it is caught seasonally [27–29], and as a commercially unavailable species, it was chosen in this research. The biology and physiology of the queen scallop have not been extensively studied, probably due to its lower market value than other commercially important species of this family, but it has received considerable attention due to its feeding mechanism and swimming ability [26]. Research and development of successful scallop breeding depend on a wide range of economic and industrial conditions. There are various initiatives for the development of Pectinidae mariculture in the United Kingdom, Spain, France, Norway, and Ireland, and to a lesser extent in Italy and Croatia [28,30,31]. Despite the development and adaptation of new technologies, scallop mariculture has not developed in Europe to date, which is mostly due to seed shortages, high production and labor costs related to the cultivation, and low population survival [32]. Nevertheless, our previous research has shown that during ex situ cultivation, queen scallops maintain good nutritional quality and can be proposed as a shellfish of choice for human consumption [20,33,34]. Furthermore, the scallops achieved the highest nutritional value during winter and spring, thus pointing to this time of year as the best period for scallop consumption.

We need to find fast ways to determine the effects of climate conditions on the environment and the laboratory setup. Today, many countries employ underwater probe instruments and corers equipped with sensors to detect the physical and chemical properties of seawater, enabling the monitoring of the aquatic environment. However, these sensors have high maintenance costs and can only detect local and short-term changes. Early environmental changes can be detected by monitoring the behavioral and physiological responses of organisms. This method allows for continuous observation and assessment of a broad spectrum of environmental alterations [35,36]. Shellfish are organisms that possess a desirable set of characteristics: wide distribution, a large number of organisms per population, commercial and mariculture value, and for this reason they are often used in studies for climate change predictions [37–42]. The most common research technique in fisheries and ecology is the analysis of individual parts of shellfish tissues and their general condition in response to changes in the seawater environment [1,43,44]. Such responses include this study of bivalve behavior, including shell growth and dissolution of their shells, premature release of larvae, or horizontal movement for some species [45]. The morphometric indices, allometric relationships, and physiological parameters are easily trackable, thus making them a good tool for the assessment of shellfish growth or lack of growth in aquaculture with changed environmental conditions [19,24,30,31,38]. Over a long period of time, many studies have used indices that can provide basic information about the state of the organism and water quality. The fitness index, growth index, and survival broadcast time comprise simple measurements that can be performed by many laboratories [36]. Methods of determining the physiological parameters of individuals, such as the effect on morphometry, allometry, and indices, are often used in scientific research on bivalves. While the queen scallop is rarely used in scientific research, these methods have provided significant insights into the cultivation of other bivalve species, such as mussels and oysters, under altered environmental conditions or in the presence of certain anthropogenic pollutants [20,46–48].

Nowadays, without a significant shift in approach and a focus on innovative, rapid, and environmentally sustainable methods for shellfish cultivation, the advancement of mariculture will face significant obstacles [49]. In addition to the discovery of a species resistant to the environmental changes ahead of us, an alternative approach was also found in the addition of a probiotic culture to mariculture. Čanak et al. [24] have shown that the probiotic strain *Lactiplantibacillus plantarum* I isolated from the queen scallop's digestive tract increased the growth rate, weight, and length of the organism and gave a significantly higher meat yield. The indigenous strain *Lpb. plantarum* I also helped the scallop growth during cultivation under stressful environmental conditions (pH 7.8, T = 16 ± 2 °C) [25,27]. Furthermore, the use and success of *Lpb. plantarum* species were recognized in crab farming, as indicated by three studies conducted on the tiger shrimp and the Pacific white shrimp [50–52]. They found that the addition of *Lpb. plantarum* bacteria to the diet significantly increased weight gain, specific growth rate, feed conversion efficiency, and protein efficiency. In this experiment, during the feeding of shrimp with probiotics, the number of lactic acid bacteria (LAB) significantly increased in the intestines, with a simultaneous decrease in the number of pathogenic bacteria. However, the number of LAB decreased after returning to the basic diet, which clearly indicates that *Lpb. plantarum* is not able to permanently colonize the intestines of Pacific white shrimp and needs to be continuously added to maintain its beneficial effect [52,53]. In aquaculture, probiotics are being increasingly used as an alternative to chemotherapy and vaccination. This approach helps to address the rise of various diseases and new strains of pathogenic microorganisms, which are emerging due to the temperature increases associated with climate change [27]. To ensure *Lpb. plantarum* I exhibits its protective benefits, it was crucial to test their strain survival under simulated climate change conditions before starting shellfish experiments, revealing that *Lpb. plantarum* I maintained a high number of viable cells even after one month [25]. Probiotics in aquaculture enhance immune function, improve nutrient utilization, and mitigate harmful algal blooms, helping shellfish resist diseases and thrive amid climate change [54].

Considering all the above, this study aims to investigate the morphological parameters of the queen scallop kept for a month ex situ in the changed environmental conditions. Furthermore, the addition of the probiotic culture *Lpb. plantarum* I to the climate change conditions was also assessed. The morphological parameters of the scallops that were analyzed in this study were: morphometric indices, condition index, meat yield, adductor muscle index, gonadosomatic index, and hepatosomatic index.

2. Materials and Methods

2.1. Scallop Culture

In February 2022, approximately 120 specimens of the queen scallop *Aequipecten opercularis* (Linnaeus, 1758) were obtained from a location approximately two nautical miles southeast of the Albanež shoal, situated within the E2 fishing zone in the Municipality of Medulin, Croatia (44°43′58.49″ N, 13°56′48.94″ E). Local fishermen collected the scallops using a bottom-trawling net deployed from a fishing vessel at a depth of around 50 m. After collection, the scallops were relocated to the Aquarium Pula facility and accommodated in a circular basin with a volume of 1900 L, featuring a flow-through seawater system with a rate of 200 L per hour. The scallops were kept in this basin during a one-week acclimatization period. A total of 90 individual queen scallops, with shell lengths averaging 51.4 ± 3.0 mm and shell widths averaging 48.5 ± 2.2 mm, were selected randomly from the 1900 L tank and utilized for this study. Throughout both the acclimatization phase and the experimental period, the scallops were provided daily nourishment, following the methodology outlined by Čanak et al. [24]. Briefly, the scallops were fed with a mixture of algae cultures comprising *Tetraselmis* sp. (Chlorophyta; 5×10^5 cells/mL), *Nannochloropsis* sp. (Eustigmatophyceae; 30×10^5 cells/mL), and *Phaeodactylum* sp. (Bacillariophyta; 12×10^5 cells/mL).

2.2. Supplementation with the Probiotic Strain Lactiplantibacillus plantarum I

The lactic acid bacteria (LAB) strain *Lactiplantibacillus plantarum* I utilized in this research was isolated from the intestinal tract of live queen scallops (*A. opercularis*), and it was thoroughly identified and characterized in our previous study [24]. This strain has been deposited in the microorganism collection of the Laboratory for General Microbiology and Food Microbiology, situated at the Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia. The *Lpb. plantarum* I strain was cultivated in MRS broth (Biolife, Milan, Italy) at 37 °C overnight, aerobically. Following, the bacterial cells were harvested through aseptic centrifugation at 6440 rcf for 10 min and washed twice with physiological saline. Subsequently, the cells were suspended in sterile physiological saline. The total viable count (TVC) of *Lpb. plantarum* I was determined using the standard dilution method on MRS agar (Biolife, Milan, Italy), with subsequent incubation at 37 °C for 24–48 h. The final count revealed 10^9 viable bacterial cells of *Lpb. plantarum* I per gram of wet biomass.

2.3. Experimental Design

After a one-week acclimatization period, three groups of 30 queen scallops were transferred into closed-system tanks with a total volume of 190 L, each having a working volume of 160 L. The tanks were aerated using single aeration stones and maintained for a duration of 30 days. Seawater within the tanks underwent continuous filtration via mechanical aquarium filters, except during feeding hours (from 9 a.m. to 3 p.m.). Every other day, 20% of the seawater was exchanged. The photoperiod was regulated to maintain 12 h of light and 12 h of darkness.

The control tank was maintained under natural seawater conditions for this period of the year (March 2022) with a stable temperature (~15 °C) and pH (~7.8), and the scallops were fed solely with algae culture. In the first experimental tank (ΔpHT), scallops were exposed to simulated climate change conditions that included a temperature increase of approximately 2 °C (~17 °C) and a pH reduction of 0.2 (7.6). In the second experimental tank (ΔpHT + LAB), scallops were exposed to the same aforementioned climate change conditions, and *Lpb. plantarum* I culture (~10^2 CFU/mL) was introduced daily during feeding. Climate change conditions were simulated according to predictions by Salgado-García et al. [1]. The environmental parameters in the tanks were changed in such a way that the pH value was reduced through a pipe that released carbon dioxide bubbles from the gas bottle into the water, which reduced the pH of the seawater in the experimental tank. The tube was built with a spiral system so that the gas bubbles slowly dissolve in the water without rising to the surface and passing into the atmosphere. The temperature was raised using an aquarium heater that constantly heated the two experimental tanks (ΔpHT and ΔpHT + LAB). Finally, in one pool with changed environmental conditions, a probiotic was added. A probiotic for shellfish was applied along with feeding as a starter culture. Algae concentration for feeding was modified based on the scallop population and *Lpb. plantarum* I culture presence. Water flow was restricted during feeding. Temperature, pH, and dissolved oxygen were monitored daily using a Hanna HI98193 multiparameter probe. Two repetitions for each condition have been conducted without any differences. The experimental design is shown in Figure 1.

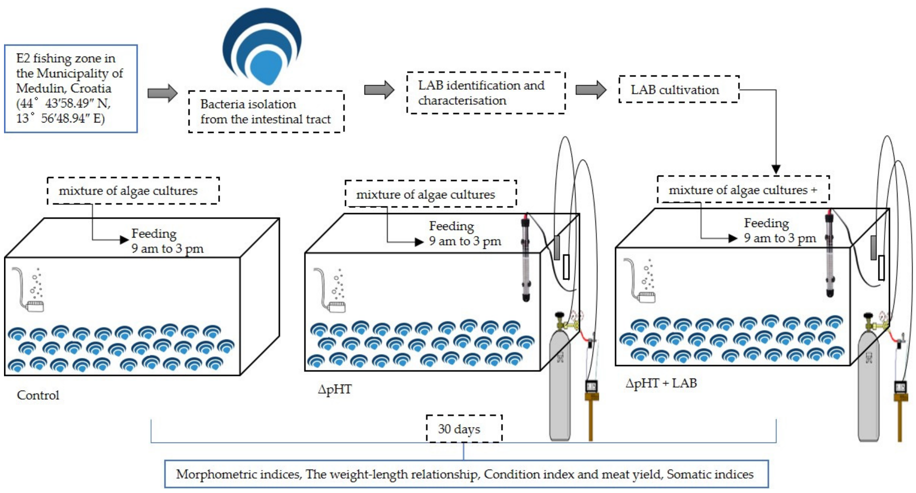

Figure 1. The illustration of the queen scallops *A. opercularis* kept for 30 days in the control conditions, exposed to changed climate conditions (ΔpHT) and changed climate conditions with the additions of *Lpb. plantarum* I (ΔpHT + LAB).

2.4. Morphological Measurements

Randomly selected 90 individuals from the 1900 L tank (where the scallops were kept during the acclimatization period) were initially cleaned of fouling so that further measurement could be performed accurately. After cleaning, the scallops were marked with different numbers to enable individual tracking before and after the experiment. Before and after a month of cultivation, the scallops were measured for different morphometric parameters, as described previously by Schmidt et al. [43]. Briefly, the shell dimensions (shell length-SL, shell height-SH, and shell width-SW) of individual specimens were assessed using a digital steel caliper with an accuracy of 0.1 mm. The total wet weight (TW) and wet meat weight (MW) of each bivalve were measured using an electronic balance. Subsequently, adductor muscle, gonads, and hepatopancreas were isolated from the dissected scallop wet meat and weighed alone. Various morphometric indices, such as elongation index (SH/SL), compactness index (SW/SL), convexity index (SW/SH), and density index (TW/SL), were utilized to characterize the morphology and growth patterns of bivalves [55–57].

The morphometric relationship was determined using the allometric equation proposed by Ricker [58], Y = a Xb, where 'Y' represents TW, while 'X' denotes the shell length (SL), 'a' stands for the intercept, and 'b' represents the slope. The allometric coefficient, expressed by the exponent 'b' in linear regression equations, indicates whether the growth is isometric ($b = 3$) or allometric ($b < 3$ or $b > 3$) [59]. To verify if the obtained 'b' values significantly deviated from the isometric value ($b = 3$) and indicated a negative ($b < 3$) or positive ($b > 3$) allometric relationship [60].

To evaluate the condition index (IC), meat yield (MY), adductor muscle index (AI), gonadosomatic index (GSI), and hepatosomatic index (HSI), the scallop's body was dissected, and the gonads and muscles were separated from the rest of the soft tissue. Condition index (CI) and meat yield (MY) were calculated using the following formulas:

$$\text{CI (\%)} = [\text{MW (g)}/\text{SW(g)}] \times 100 \text{ and}$$

$$\text{MY (\%)} = [(\text{MW (g)}/\text{TW (g)}) \times 100].$$

Somatic indices: adductor muscle index (AI), gonadosomatic index (GSI), and hepatosomatic index (HSI) were calculated as follows:

$$\text{AI (\%)} = [\text{adductor muscle wet weight (g)}/\text{wet body weight (g)}] \times 100,$$

$$\text{GSI (\%)} = [\text{gonad wet weight (g)}/\text{adductor muscle wet weight (g)}] \times 100, \text{ and}$$

$$\text{HSI (\%)} = [\text{hepatopancreas wet weight (g)}/\text{adductor wet weight (g)}] \times 100.$$

2.5. Statistical Analyses

Morphometric indices, condition indices, meat yield, and somatic indices were subjected to a one-way ANOVA to test the effect of climate change conditions and the effect of *Lpb. plantarum* I on the climate change conditions on the 30th day of the experiment. When significant differences were found, the mean values were subjected to post hoc analysis using Tukey's HSD test ($p < 0.05$). To test the difference between morphometric indices measured at the beginning (1st day) and at the end (30th day) of the experiment, Tukey's HSD test was used ($p < 0.05$). Analysis of covariance (ANCOVA) was applied to determine the difference in the log transformed data of the weight-length relationship between control scallops and scallops in climate change conditions and scallops with the effect of *Lpb. plantarum* I in the climate change conditions. All analyses were performed using the Statistica 9.0 software (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Seawater Conditions

During the experiment, the average temperature in the control tank was recorded to be 15.67 ± 0.65 °C, and the pH was 7.88 ± 0.07. In the ΔpHT tank, the average temperature was 16.88 ± 0.46 °C and the pH was 7.64 ± 0.08, while in the ΔpHT + LAB tank, the average temperature was 16.83 ± 0.49 °C and the pH was 7.64 ± 0.08. In all three experimental tanks, the dissolved oxygen concentration was stable at ~90% (Table 1).

Table 1. Temperature, pH, and dissolved oxygen levels (mean ± SD) of the seawater measured in the control tank with no changed conditions (Control), in the experimental tank where the climate conditions were changed (ΔpHT), and in the experimental tank where the climate conditions were changed and the strain *Lpb. plantarum* I was added as a food supplement (ΔpHT + LAB).

	Control	**ΔpHT**	**ΔpHT + LAB**
pH	7.88 ± 0.07	7.64 ± 0.08	7.67 ± 0.08
Temperature (°C)	15.67 ± 15.65	16.88 ± 0.46	16.83 ± 0.49
Dissolved oxygen (%)	89.94 ± 1.59	89.92 ± 1.54	89.94 ± 1.50

3.2. Morphometric Indices, Allometry, and Somatic Indices

The elongation index (EI) of the scallops kept in the Control tank remained the same during the experimental period of 30 days (EI = 0.987 ± 0.019) (Table 2). In the ΔpHT tank, a slight increase (1.012 ± 0.083) was recorded in the EI of the scallops on the 30th day of the experiment. Whereas, in the ΔpHT + LAB experimental tank, the EI was again the same as on the 1st day of the experiment (EI = 0.98 ± 0.014) and very similar to the one recorded in the control scallops. The compactness index (CMI) was found to be statistically different on the 30th day of the experiment only in the ΔpHT tank (CMI 1st day = 0.321 ± 0.018; CMI 30th day = 0.337 ± 0.027). On the other hand, the control tank and the ΔpHT + LAB tank showed no statistically significant differences between the 1st and 30th days of the experiment. The obtained density index (DI) was recorded to be lower if comparing the 1st and last days of the experiment again only for the ΔpHT tank (DI 1st day = 0.288 ± 0.032; DI 30th day = 0.256 ± 0.030). The morphometric parameter convexity index (CVI) showed no differences in the duration of the experiment for all tested tanks.

Table 2. Morphometric indices (mean ± SD) measured for the queen scallops *A. opercularis* on the 1st and 30th day of the experiment kept in different tanks: in the control tank, exposed to pH and temperature change conditions (ΔpHT), and exposed to pH and temperature change conditions with the addition of *Lpb. plantarum* I (ΔpHT + LAB). Bolded values are significantly different from the first day of exposure (Tukey HSD test, $p < 0.05$).

	Control	**ΔpHT**	**ΔpHT + LAB**
Elongation index			
1st day	0.987 ± 0.019	0.988 ± 0.017	0.98 ± 0.017
30th day	0.987 ± 0.019	1.012 ± 0.083	0.98 ± 0.014
Compactness index			
1st day	0.335 ± 0.023	0.321 ± 0.018	0.330 ± 0.018
30th day	0.338 ± 0.020	**0.337 ± 0.027**	0.337 ± 0.023
Convexity index			
1st day	0.339 ± 0.024	0.325 ± 0.019	0.332 ± 0.020
30th day	0.343 ± 0.022	0.334 ± 0.018	0.341 ± 0.024
Density index			
1st day	0.309 ± 0.038	0.288 ± 0.032	0.290 ± 0.032
30th day	0.292 ± 0.040	**0.256 ± 0.030**	0.283 ± 0.043

The values of the allometric coefficient b were similar for all scallops on the 1st day of the experiment and varied between 2.221 (ΔpHT) and 2.991 (ΔpHT + LAB), indicating a slight negative allometry. The weight-length relationship on the 30th day of the experiment decreased in scallops in climate change conditions (ΔpHT) but increased in control scallops and scallops from the ΔpHT + LAB experimental tank ($p < 0.05$) (Table 3).

Table 3. The weight-length relationship: Allometry (A+, positive; A−, negative), a-intercept, and b-slope (mean ± SD) measured for the queen scallops *A. opercularis* kept in the control tank (Control), the tank with the changed pH and temperature change conditions (ΔpHT), and in the tank with pH and temperature change conditions with the addition of *Lpb. plantarum* I (ΔpHT + LAB). Bolded values are significantly different from the first day of the experiment (ANCOVA, $p < 0.05$).

	Control	**ΔpHT**	**ΔpHT + LAB**
a			
1st day	2.302	2.433	2.462
30th day	2.532	2.942	2.925
b			
1st day	2.707	2.221	2.991
30th day	3.125	**0.943**	**3.798**
Allometry			
1st day	A−	A−	A−
30th day	**A+**	**A−**	**A+**

The condition indices (CI) and the meat yield (MY) did not show any statistical differences among all three tested tanks (Figure 2). Nevertheless, a slight increase in CI and MY was noted when comparing the scallops from the control tank to the other two tested tanks. The CI was noted to be 37.34 ± 10.20% for the scallops kept in the control tank. The CI for the scallops kept in the ΔpHT tank was 40.54 ± 8.19%, while the same parameter was found to be 41.36 ± 4.42% in the ΔpHT + LAB tank. Further, the MY for the scallops kept for a month in the control tank was recorded to be 18.60 ± 4.29%. The MY was higher in the other two tanks, with values of 21.18 ± 3.39% in the scallops exposed to ΔpHT and 20.81 ± 2.36% in the scallops exposed to ΔpHT + LAB tank.

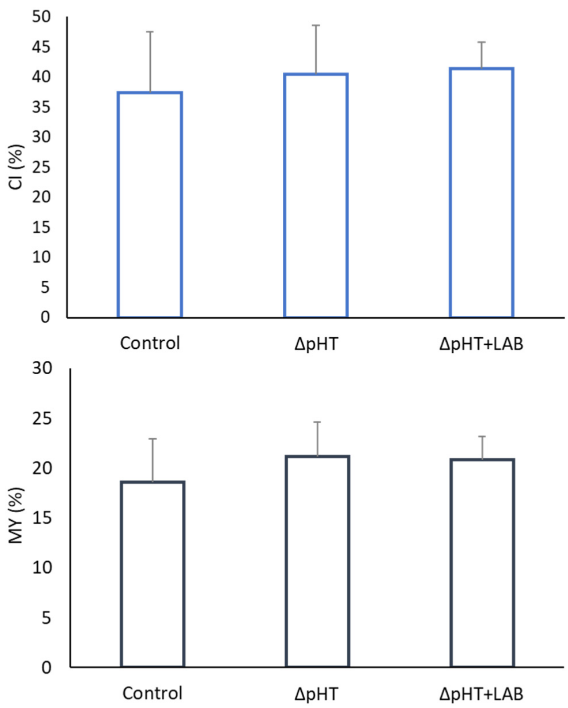

Figure 2. Condition index (CI, %) and meat yield (MY, %) of the queen scallops *A. opercularis* kept for 30 days in the control conditions, exposed to changed climate conditions (ΔpHT), and changed climate conditions with the addition of *Lpb. plantarum* I (ΔpHT + LAB).

In Figure 3, the adductor muscle index (AI), gonadosomatic index (GSI), and hepatosomatic index (HSI) are presented for all three experimental tanks. The AI for the scallops kept in the control tank for 30 days was recorded to be the highest (30.72 ± 14.95%). No statistical difference in the AI was noted between the scallops kept in the control tank and the scallops kept in the changed climate conditions (ΔpHT). Contrary to this, the AI obtained for the scallops kept in the ΔpHT + LAB tank was found to be significantly lower compared to the control tank (AI = 6.80 ± 6.73%; $p < 0.05$). The GSI was noted to be very similar in the scallops kept in the control conditions (3.92 ± 3.33%) and the ones kept in the changed climate conditions (3.58 ± 1.00%). On the other hand, the GSI was statistically significantly higher in the scallops kept in the ΔpHT + LAB tank (10.22 ± 10.02%, $p < 0.05$). Finally, the HSI showed no difference among all tested scallops and ranged from 10.28 ± 2.02 up to 10.75 ± 5.78% for all 90 scallops included in the 30-day experimental design.

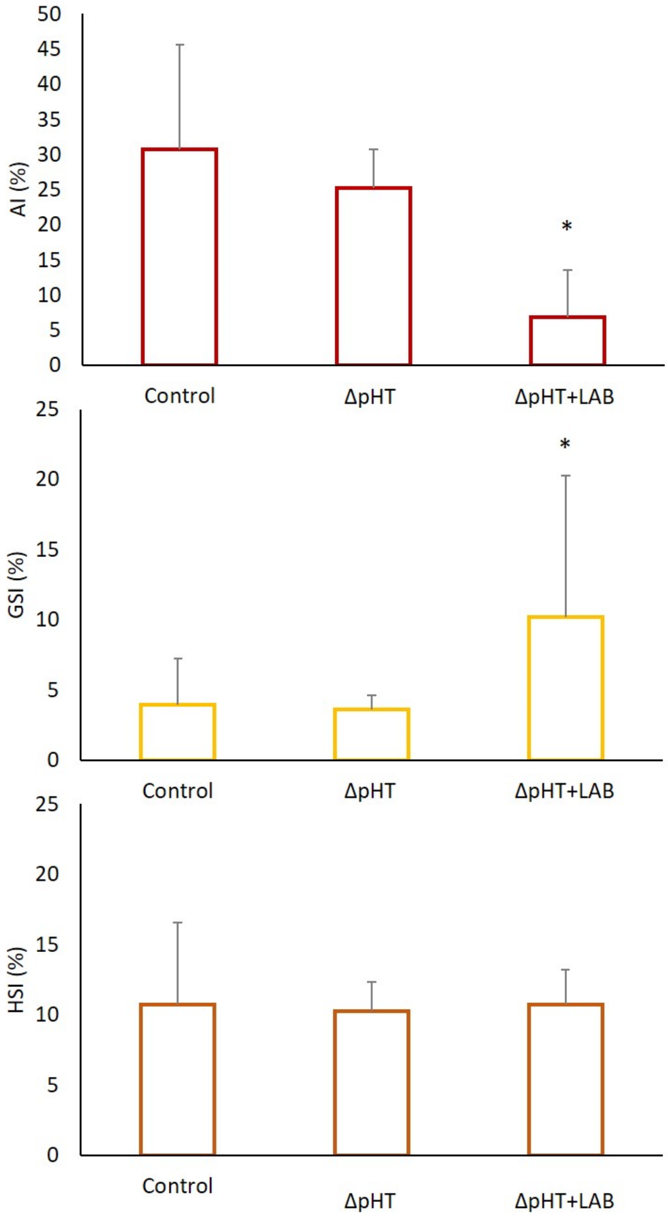

Figure 3. Adductor muscle index (AI, %), gonadosomatic index (GSI, %), and hepatosomatic index (HSI, %) of the queen scallops kept for 30 days in the control conditions, exposed to changed climate conditions (ΔpHT) and changed climate conditions, and the additions of *Lpb. plantarum* I (ΔpHT + LAB). The means labeled with the asterisk are significantly different (Tukey's HSD test, $p < 0.05$).

4. Discussion

Climate change exerts significant pressure on coastal ecosystems by altering water biogeochemical and physicochemical parameters (e.g., temperature, pH, and salinity), ultimately leading to the degradation of aquatic ecosystems [4,9–13]. Accordingly, in the future climate change scenario model for years 2070 and 2100, it was pointed out that salinity and temperature would be the main drivers of changes, together with macronutrients, especially in the area of the Po' River delta in the North Adriatic [9]. Moreover, the increase in sea temperature and the decrease in pH value can have negative consequences, such as an increase in coral bleaching [10] and a change in marine species abundance [11].

Due to the negative impact of climate change on aquaculture, there is an inevitable need to develop research to breed shellfish strains that are more resistant to climate change. Such an approach is crucial for optimizing shellfish aquaculture production in light of climate change [61]. Many studies have been conducted with the aim of producing high-quality foods that provide a rich source of human nutrition and economic value for local communities [20,33,62]. Since the resistance of bivalves to climate change is low, intensive work is being performed in selective breeding to create bivalve strains that are more resistant to such conditions and to find solutions for new environmental conditions that may occur in the near future [20,24,25,27,33,63]. Furthermore, the increase in sea temperature and the decrease in pH value can have negative consequences, such as an increase in the number of pathogens (bacterial and viral) that survive better at higher temperatures because of their faster division rates, which can ultimately lead to the death of entire shellfish populations [25,27].

Considering all the above, this study was designed as a response to continuous challenges for the long-term sustainability of the mariculture sector and the safety and health of consumers of marine products under climate change conditions. The main goal of this research was to observe the impact and vulnerability of living bivalves due to climate change and the possibilities of adaptation of certain species to such changes. During the experiment, changes were applied simultaneously to the temperature and pH values in the tanks where the bivalves were kept to determine the effect of the upcoming climate changes on the marine environment. The chosen parameters (T and pH) were determined in our previous research to be the most sensitive to the growth of the scallops and were tried to be simulated as conditions of global warming ex situ [20,24,25,33]. In this research, an increase in temperature of 2 °C, a decrease in pH value of 0.2 units, and the addition of probiotic culture *Lpb. plantarum* I were tested on the growth and development of the scallops (morphometry, allometry, and fitness indices) during the 30-day experiment. In the control tank, temperature and pH represented the characteristic values of these parameters found at the place from where the scallops were sampled in the sea and their normal fluctuations throughout the spring period in the Adriatic Sea [20,34].

Different morphometric indices, such as elongation index (SH/SL), compactness index (SW/SL), convexity index (SW/SH), and density index (TW/SL), were utilized in this study to characterize the morphology and growth patterns of the scallops in the environmentally changed conditions and with the addition of the probiotic culture. These indices have already been used in numerous studies and have been proven to be good tools for examining the growth of the bivalves [55–57]. The morphometric indices of all scallops used in this study gave a similar value with no statistical difference on the first day of the experiment for all three tanks, indicating the uniformity of the selected population of scallops in the environment. Furthermore, the results noted for the morphometric indices of scallops kept in the control tank indicate that the four tested indices do not change during the period of one month if the scallops are kept in environmentally relevant climate conditions. The elongation index (EI) and the convexity index (CVI) did not show any significant change during the experimental design in all three set-up tanks, with only a slight increase in the EI for the scallops that were kept in the ΔpHT tank. These results point out that at this time of year, the scallops do not grow in height and length but are most likely to put all their energy into gonadal development due to the upcoming spawning period in spring. This hypothesis can be further confirmed with a higher GSI index also recorded in this study, especially for the ΔpHT + LAB tank (GSI = 10.22%). The highest GSI (34.06%) was already recorded for queen scallop *A. opercularis* in the spring period if the scallops were kept in captivity under controlled environmental parameters [20]. Differences in the morphometric indices were noted for the compactness index (CMI) and the density index (DI), which were both statistically different in the ΔpHT tank on the 30th day of the experiment if compared to the first day. The CMI was found to be elevated in the scallops that were kept in the ΔpHT tank for a month. The higher CMI could be due to a process called hormesis, in which, under mild stress conditions, the organism tends to recuperate and express better survival than in normal conditions [64]. This result makes them a good alternative species for aquaculture in the upcoming change in sea temperature and pH since it was noted that the scallops can adapt quickly to climate change conditions by increasing compactness and density, thus preventing the negative effects of the changed environmental conditions. Ocean acidification is considered the greatest threat to calcifying organisms; however, recent research suggests the need to reconsider this concept. More than 70% of this research on the effects of acidification on growth and calcification indicates the ability of the tested organisms to adapt. Fernandez-Reiriz et al. [38] conducted a study on the effects of acidification on the physiology of the bivalve *Mytilus galloprovincialis* (Lamarck, 1819) and concluded that the maximum extent of growth was observed in individuals exposed to reduced pH values (−0.3 and −0.6 pH units as compared with the control seawater). In the aforementioned study, the species showed adaptation to acidification and increased ammonia excretion as an intracellular pH regulation mechanism. It has been confirmed that growth, absorption efficiency, and ammonia excretion increase with decreasing pH values. Mussels respond to acidification by allocating stored energy to preserve somatic tissue, with negative consequences for shell integrity that vary among species [65]. Contrary to the results obtained in this study for the CMI, the DI was found to be lower in the ΔpHT tank compared to the 1st day of the experiment set up. The lower DI was expected for the ΔpHT tank since it was expected that the change in climate conditions would result in a reduction of the morphometric parameters of the scallops kept ex situ for a month. Furthermore, an interesting effect was observed in the tank where scallops were given a food supplement containing the strain *Lpb. plantarum* I. It appears that the addition of the LAB culture masks the change introduced by the higher temperature and lower pH. This indicates that the LAB culture is compensating for the stress that the ΔpHT alone is causing on the scallops if CMI and DI indices are considered. Namely, the results of the morphometric indices for the control and the ΔpHT + LAB tank were the same for all tested morphometric indices.

The concept of allometry—this study of how body proportions change with size—plays a crucial role in understanding the responses of organisms to various environmental conditions, including climate change [31,41,44,66]. In the context of climate change, positive allometry implies that certain body parts or traits increase disproportionately in size compared to overall body size as environmental conditions shift. Conversely, negative allometry suggests that these traits grow at a slower rate relative to overall body size. Numerous studies have investigated the allometric growth patterns of mussels and other bivalves in different regions, particularly in response to changing environmental conditions. Scallops in the Gulf of Taranto, the Ionian Sea, Southern Italy [31], and the Aegean Sea coast of Turkey [66] have all employed regression analysis to determine the growth patterns of bivalves in various habitats. Their findings consistently indicate negative allometry, implying that certain traits of mussels grow at a slower rate compared to overall body size. Furthermore, recent research conducted in Çanakkale Strait (Turkey) in 2023 also supports these observations, revealing that many bivalve species exhibit negative allometry in their growth patterns [44]. This aligns with previous findings and underscores the importance of considering allometric relationships when studying the impacts of climate change on marine organisms. The prevalence of negative allometry in bivalves underlines the complex interplay between environmental factors, such as temperature, nutrient availability, habitat characteristics, and organismal growth patterns [41]. Understanding these relationships is critical for predicting how bivalve populations may respond to ongoing climate change and its associated challenges, including ocean acidification and shifts in habitat suitability. In our study, scallops with the addition of probiotic culture (*Lpb. plantarum* I) showed positive allometry, which may indicate certain adaptive responses to climate change in scallop species and may suggest them as potential alternative aquaculture species in the Adriatic Sea in the near future.

The condition index (CI), meat yield (MY), and gonadosomatic index (GSI) are important parameters for evaluating the quality of shellfish meat [43]. In this study, the scallops kept in the ΔpHT and ΔpHT + LAB tanks showed a slight increase in condition index (CI) and meat yield (MY) compared to the values obtained from the scallops kept in the control tank. However, the difference between the tanks was not found to be statistically significant. The obtained results point out that the duration of the experiment over a period of 30 days might be crucial for these parameters to show an effect on this specific bivalve species. This assumption is further supported by one of our previous studies, where the scallop *A. opercularis* showed statistically significant differences in the CI and MY during a year-long experiment in controlled conditions in captivity [20]. However, the effect of adding a probiotic culture to the diet would be more likely to be noticeable, i.e., probiotic compensation for stressful environmental conditions. This remains to be investigated in future research since we have previously proven that the scallops can be kept in aquaculture for a year, during which period they maintain their commercial value [20,33]. Another explanation for the obtained results could be the selection of the time of year for this experiment, which took place in March. Namely, at this time of the year, the bivalves do not grow but rather invest all their energy in the growth of gonads due to the upcoming spawning period in spring. In this research by Kovačić et al. [20], the CI for the queen scallop *A. opercularis* in the spring was noted to be approximately 70%, a result that is almost two times higher than the CI of the scallops obtained in this study for the control tank (CI = 37.34). Contrary to our results, warming and acidification resulted in a decrease in the nutritional value of the sea snail *Dicathais orbita* (Gmelin, 1791), especially protein content and MY, which scientists attributed to the cumulative impact of environmental factors [67]. Furthermore, in many studies, CI was found to be altered by the climatic conditions. In a study conducted on mussels (*Mytilus edulis* and *M. galloprovincialis*) and oysters (*Crassostrea gigas* and *Ostrea edulis*) ex situ for a month, the CI was higher at higher food concentrations and decreased with increasing temperature [42]. Similar results were obtained in another study where the effect of climate change was studied on the bivalve *Cerastoderma edule* (Linnaeus, 1758) in laboratory conditions, and the results showed that the combined effects of warming and acidification had the greatest impact on the CI [40]. The results of the aforementioned research indicate a synergistic effect of two parameters on the CI. The impact of the combined factors of lowered pH and elevated temperature was also investigated on the growth and strength of mussel shells [39]. Acidification and warming resulted in a decrease in shell strength and a drop in the CI, and a significant difference was observed in comparison to the control pool that contained seawater with parameters equal to marine environmental conditions. Contrary to these findings, a study showed that the growth of 47 Atlantic and Mediterranean sardine *Sardina pilchardus* populations was not related to the increase in sea temperature but was recorded to be positively related to oxygen levels. These results point out that sardines grow larger in size in a more oxygenated marine environment [12].

The adductor muscle index (AI) for the scallops kept in the ΔpHT + LAB tank was found to be significantly lower ($p < 0.05$) compared to the control tank. On the other hand, the gonadosomatic index (GSI) was recorded to be significantly higher in the same tank, thus indicating that the group of scallops kept in the ΔpHT + LAB tank compensates for the lower AI by building the gonads for the upcoming spawning period during spring. This could pose a great advantage for scallops in the marine environment with the upcoming change in environmental conditions in the near future. Since it appears that only with the addition of the strain *Lpb. plantarum* I in the changed climate conditions, the scallops almost duplicated their gonad mass. The breeding season for the scallops starts in April and lasts until September in the Mediterranean Sea. The highest reproduction rate occurs in mid-summer in the Adriatic Sea, especially in July and August [20]. In the same period, with a maximum in July and August, the spawning season is reported for other shellfish species, such as the oyster *Crassostrea gigas* [68]. As breeding time approaches, the scallops concentrate growth and development into the gonads so that they are ready to go through gametogenesis and gamete formation by summer. At the same time, due to the growth of the gonads, the mass of other soft tissues, including the scallop adductor muscles, stagnates because the energy for growth stops being consumed. Another explanation could be that the AI was much lower in scallops exposed to changed climatic conditions and the addition of a probiotic culture because the shellfish sensed the presence of a large volume of food (i.e., an increase in probiotic cultures in their surroundings). Therefore, the bivalves kept their shells open for a long time, which could have led to the weakening of the adductor muscle, i.e., its total mass, throughout the month of the experiment.

The growth and reproduction of shellfish depend on various biotic and abiotic conditions, such as food availability, environmental conditions, and the metabolic needs of bivalves. In many previous studies, an increase in temperature is cited as the main factor that affects the reproduction of bivalves in the form of an increase in GSI [69–71]. If the food concentrations are optimal, energy for gametogenesis in shellfish is taken directly from the food [72]. However, in one study that investigated the influence of temperature on GSI, it was determined that the availability of food is one of the critical factors that affect the maturation of the gonads, where individuals under the influence of temperature and not fed showed lower values of the index in contrast to fed individuals [70]. Bivalves usually store glycogen as reserve energy in the adductor, which they use for gametogenesis. In such cases, the AI varies inversely proportional to the GSI, and during reproduction, the muscle loses weight. Similar results were obtained by Pichaud et al. [73], who concluded that gametogenesis occurs when the food is of sufficient quality to support the energy requirements of various processes, and when the food source is insufficient, reserve energy is used in the bivalve adductor. In another study, scientists linked the increase in the total mass of the scallop *Placopecten magellanicus* (Gmelin, 1791) to better gonadal development with feeding that had a large effect on growth at higher temperatures [74]. In the aforementioned research, the change in temperature itself did not prove to be significant for the growth rate and mass of bivalves, but for the high or low nutrition they received ex situ. Likewise, in this study, the addition of probiotic culture had a significant positive effect on the GSI of the scallops.

Finally, the hepatosomatic index (HSI) did not differ between all 90 scallops used in this study, non-depending on the experimental tank, thus not giving any data regarding the possible positive effect of the addition of probiotic culture in the changed climate conditions.

To sum up the obtained data from this study, we hypothesized that the change in temperature and pH conditions would contribute to a lower condition index and meat yield of scallops compared to those that were kept in the control tank and that the addition of probiotics would improve their growth. However, according to the obtained results, it is undeniable that such changed climatic conditions do not have a negative effect on the CI and MY of the scallop *A. opercularis* kept in cultivation ex situ for a month. These results further confirm the choice of the queen scallop as an alternative species that is suitable for successful survival in the conditions of climate change. This type of bivalve is limited to warmer waters; therefore, it occupies waters whose temperature ranges from an average winter minimum of 5 °C to an average summer maximum of 24 °C. Due to its wide temperature tolerance, it is likely that the scallop, *A. opercularis*, is capable of adapting to this variability in temperature [37]. A positive correlation between organism growth, temperature increase, and pH decrease is visible in another study conducted on the sea star *Pisaster ochraceus* [48]. In this study, the individuals were separately exposed to water temperatures ranging from 5–21 °C. The increase in temperature caused a linear increase in the growth rate of the organism and accelerated the feeding rate, with an optimum between 15 and 20 °C. Although the relative calcified mass of *P. ochraceus* decreased with increasing CO_2, the overall growth rate was not changed. These results are explained by the possibility that the calcified part of this organism makes up a relatively small proportion of its total mass. Another possible explanation that the authors mentioned is that reduced pH increases feeding efficiency. For example, the slightly lower pH of seawater could help digest prey tissues and make feeding less energetically demanding. It is emphasized that the increase of these two parameters will not have direct negative effects on all marine invertebrates, the same statement that can be concluded from this research as well. Contrary to these findings, adult mussel *M. galloprovincialis* was found to be very sensitive to sea warming, with 100% mortality observed at an elevated temperature during the summer of +3 °C above the upper critical temperature limit for mussels (25–28 °C) [46]. Furthermore, in another study, the sea acidification and increase in temperature resulted in a negative effect on the juvenile fluted giant clam *Tridacna squamosa*, which showed a much lower survival rate of <20% when a reduced pH (7.83) was combined with an increased temperature (~30 °C) [47]. Although this species mostly inhabits the warm seas of the South Pacific and Indian Oceans, it does not show a good possibility of survival under the conditions of global warming. Most of this research to date is based on already commercial bivalve species, as in the aforementioned study on *M. galloprovincialis*; thus, the detection of species whose adaptation might be possible under stressful conditions, such as the scallop *A. opercularis*, is of the greatest advantage.

This study has proven that queen scallops adapt well to the changing environmental conditions that lie ahead. Due to climate change happening nowadays, it is necessary to start the aquaculture of these shellfish in the Adriatic in the near future. Moreover, since the increase in temperature was recorded for the Adriatic Sea [5–8], future research should examine the effect of the temperature increase on the queen scallops in vivo in mariculture cultivation. In the aforementioned study, incorporating a probiotic culture as a nutritional supplement would be advantageous to monitor not only the growth indices of the bivalve but also the immune response of the shellfish. Furthermore, it would be interesting to examine how the probiotic culture is absorbed in the digestive system of this bivalve.

5. Conclusions

To date, a lot of research has been conducted examining the effect of abiotic parameters (especially T and pH) on marine organisms, including bivalve species. However, in such research, a proposal for adapting to new environmental conditions is rarely offered. Namely, in this research, it was proven that the change of environmental parameters does not have a negative effect on the scallop and that the addition of probiotic culture induces the growth of gonads in the climate-changed conditions. The obtained results point to the need for additional and more extensive research in the field of the use of lactic acid probiotics as a supplement for shellfish mariculture, especially in examining the state of oxidative stress in scallops. To overcome the stressful effects of the temperature increase and pH decrease in mariculture in the near future, we propose two main solutions: alternative species with good adaptations to new environmental conditions and the addition of probiotics isolated from the same organism.

To conclude, the results from this study point out that the addition of *Lpb. plantarum* I to the scallop *A. opercularis* diet enhances the growth of the scallop gonads in the environmentally changed climate conditions in captivity.

Author Contributions: Conceptualization, I.K., J.F. and M.Š.; Methodology, I.K. and I.Č.; Formal Analysis, I.K., P.B., A.Ž., N.I., A.P, V.K. and I.Č.; Investigation, I.K., P.B., A.P. and V.K.; Resources, J.F. and M.Š.; Writing—Original Draft Preparation, P.B.; Writing—Review and Editing, M.Š. and I.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the European Regional Development (KK.05.1.1.2. 0012) in the framework of the project "Integrated system of shellfish farming under climate change conditions" (project led by Prof. Jadranka Frece, PhD).

Institutional Review Board Statement: No ethical review was necessary in the Croatia for work with mussels as they are invertebrates.

Data Availability Statement: The data that supports this research are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Salgado-García, R.L.; Kraffe, E.; Maytorena-Verdugo, C.I.; Rivera-Camacho, A.R.; Sicard, M.T.; Arellano-Martínez, M.; Racotta, I.S. Metabolic responses of adult lion's paw scallops *Nodipecten subnodosus* exposed to acute hyperthermia in relation to seasonal reproductive effort. *Sci. Rep.* **2020**, *10*, 2449. [CrossRef]
2. Soon, T.K.; Zheng, H. Climate Change and Bivalve Mass Mortality in Temperate Regions. *Rev. Environ. Contam. Toxicol.* **2019**, *251*, 109–129. [CrossRef]
3. Mahato, A. Climate change and its impact on agriculture. *Int. J. Sci. Res.* **2014**, *4*, 1–6.
4. Gentilucci, M.; Parisi, C.; Coppola, M.R.; Majdoubi, F.-Z.; Madonna, A.; Guerriero, G. Influence of Mediterranean Sea Temperature Increase on Gaeta Gulf (Tyrrhenian Sea) Biodiversity. *Proc. Zool. Soc.* **2021**, *74*, 91–103. [CrossRef]
5. Grbec, B.; Matić, F.; Beg Paklar, G.; Morović, M.; Popović, R.; Vilibić, I. Long-Term Trends, Variability and Extremes of In Situ Sea Surface Temperature Measured Along the Eastern Adriatic Coast and Its Relationship to Hemispheric Processes. *Pure Appl. Geophys.* **2018**, *175*, 4031–4046. [CrossRef]
6. Bonacci, O.; Vrsalović, A. Differences in Air and Sea Surface Temperatures in the Northern and Southern Part of the Adriatic Sea. *Atmosphere* **2022**, *13*, 1158. [CrossRef]
7. Vilibić, I.; Dunić, N.; Peharda, M. Near-Surface Ocean Temperature Variations across Temporal Scales in the Coastal Eastern Adriatic. *Cont. Shelf Res.* **2022**, *245*, 104786. [CrossRef]
8. Raicich, F.; Colucci, R.R. A near-surface sea temperature time series from Trieste, northern Adriatic Sea (1899–2015). *Earth Syst. Sci. Data* **2019**, *11*, 761–768. [CrossRef]
9. Rizzi, J.; Torresan, S.; Critto, A.; Zabeo, A.; Brigolin, D.; Carniel, S.; Pastres, R.; Marcomini, A. Climate change impacts on marine water quality: The case study of the Northern Adriatic sea. *Mar. Pollut. Bull.* **2016**, *102*, 271–282. [CrossRef]
10. Kružić, P.; Popijač, A. Mass mortality events of the coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae) in the Mljet National Park (eastern Adriatic Sea) caused by sea temperature anomalies. *Coral Reefs* **2015**, *34*, 109–118. [CrossRef]
11. Pavičić, M.; Vilibić, I.; Šepić, J.; Vrdoljak, D.; Stagličić, N.; Šegvić Bubić, T.; Vujević, A.; Matić-Skoko, S. Temperature-driven abundance change of European lobster (*Homarus gammarus*) in the Adriatic Sea. In *13. Croatian Biological Congress with International Participation—Collection of Abstracts*; Croatian Biological Society: Zagreb, Croatia, 2018; pp. 308–309. Available online: https://www.croris.hr/crosbi/publikacija/prilog-skup/693298 (accessed on 24 April 2024).
12. Dimarchopoulou, D.; Tsikliras, A.C. Linking growth patterns to sea temperature and oxygen levels across European sardine (*Sardina pilchardus*) populations. *Environ. Biol. Fish* **2022**, *105*, 1335–1345. [CrossRef]
13. Chatzimentor, A.; Doxa, A.; Katsanevakis, S.; Mazaris, A.D. Are Mediterranean marine threatened species at high risk by climate change? *Glob. Chang. Biol.* **2023**, *29*, 1809–1821. [CrossRef]
14. Scanes, E.; Byrne, M. Warming and hypoxia threaten a valuable scallop fishery: A warning for commercial bivalve ventures in climate change hotspots. *Glob. Chang. Biol.* **2023**, *29*, 2043–2045. [CrossRef]
15. Mohamed, K.S. Probiotics and its application in mariculture. In *U: Winter School on Recent Advances in Mariculture Genetics and Biotechnology, 4–24 November 2003*; Central Marine Fisheries Research Institute: Kochi, India, 2003.
16. Prado, S.; Romalde, J.L.; Barja, J.L. Review of probiotics for use in bivalve hatcheries. *Vet. Microbiol.* **2010**, *145*, 187–197. [CrossRef] [PubMed]
17. Kanduč, T.; Šlejkovec, Z.; Falnoga, I.; Mori, N.; Budič, B.; Kovačić, I.; Pavičić-Hamer, D.; Hamer, B. Environmental status of the NE Adriatic Sea, Istria, Croatia: Insights from mussel *Mytilus galloprovincialis* condition indices, stable isotopes and metal(loid)s. *Mar. Pollut. Bullet.* **2018**, *126*, 525–534. [CrossRef]
18. Topić Popović, N.; Beer Ljubić, B.; Strunjak-Perović, I.; Babić, S.; Lorencin, V.; Jadan, M.; Čižmek, L.; Matulić, D.; Bojanić, K.; Čož-Rakovac, R. Seasonal antioxidant and biochemical properties of the Northern Adriatic *Pecten jacobaeus*. *PLoS ONE* **2020**, *15*, e0230539. [CrossRef]
19. Kovačić, I.; Pavičić-Hamer, D.; Kanduč, T.; Hamer, B. Adaptation of cultured mussel *Mytilus galloprovincialis* Lamarck, 1819 from the northern Adriatic Sea to nearby aquaculture sites and translocation. *Acta Adriat.* **2017**, *58*, 285–296. [CrossRef]
20. Kovačić, I.; Žunec, A.; Mateŝković, M.; Burić, P.; Iveša, N.; Štifanić, M.; Frece, J. Commercial Quality, Biological Indices and Biochemical Composition of Queen Scallop *Aequipecten opercularis* in Culture. *Fishes* **2023**, *8*, 48. [CrossRef]
21. Filgueira, R.; Guyondet, T.; Comeau, L.A.; Tremblay, R. Bivalve aquaculture-environment interactions in the context of climate change. *Glob. Chang. Biol.* **2016**, *22*, 3901–3913. [CrossRef]
22. Melaku Canu, D.; Solidoro, C.; Cossarini, G.; Giorgi, F. Effect of global change on bivalve rearing activity and the need for adaptive management. *Clim. Res.* **2010**, *42*, 13–26. [CrossRef]
23. Gosling, E. *Bivalve Molluscs: Biology, Ecology and Culture*; John Wiley and Sons: Hoboken, NJ, USA, 2008; Volume 456.

24. Čanak, I.; Kovačić, I.; Žunec, A.; Jakopović, Ž.; Kostelac, D.; Markov, K.; Štifanić, M.; Burić, P.; Iveša, N.; Frece, J. Study of the Impact of *Lactiplantibacillus plantarum* I on the Health Status of Queen Scallop *Aequipecten opercularis*. *Appl. Sci.* **2023**, *13*, 7723. [CrossRef]
25. Čanak, I.; Kovačić, I.; Žunec, A.; Jakopović, Ž.; Kostelac, D.; Markov, K.; Štifanić, M.; Burić, P.; Iveša, N.; Frece, J. Effect of dietary supplementation with *Lactiplantibacillus plantarum* i on queen scallop *Aequipecten opercularis* under simulated climate change conditions. *Croat. J. Fish.* **2024**, *82*, 1–8. [CrossRef]
26. Duncan, P.F.; Brand, A.R.; Strand, Ø.; Foucher, E. The European scallop fisheries for *Pecten maximus*, *Aequipecten opercularis*, *Chlamys islandica*, and *Mimachlamys varia*. *Dev. Aquac. Fish. Sci.* **2016**, *40*, 781–858. [CrossRef]
27. Čanak, J.; Kostelac, D.; Jakopović, Ž.; Markov, K.; Frece, J. Lactic Acid Bacteria of Marine Origin as a Tool for Successful Shellfish Farming and Adaptation to Climate Change Conditions. *Foods* **2024**, *13*, 1042. [CrossRef]
28. Marčeta, T.; Marin, M.G.; Codognotto, V.F.; Bressan, M. Settlement of Bivalve Spat on Artificial Collectors (Net Bags) in Two Commercial Mussel Parks in the North-Western Adriatic Sea. *J. Mar. Sci. Eng.* **2022**, *10*, 210. [CrossRef]
29. Šimunović, A.; Piccinetti, C.; Despalatović, M.; Grubelić, I. Experimental catches and distribution of Queen scallop *Aequipecten opercularis* (LINNAEUS, 1758) (Pectinidae, Mollusca Bivalvia) in the Adriatic Sea. *Acta Adriat.* **2002**, *43*, 49–57.
30. Rathman, M.; Bolotin, J.; Glavić, N.; Barišić, J. Influence of water depth on growth and mortality of *Chlamys varia* (Linnaeus, 1758): Implications for cage culture in Mali Ston Bay, Croatia. *Aquac. Int.* **2017**, *25*, 135–146. [CrossRef]
31. Prato, E.; Biandolino, F.; Parlapiano, I.; Papa, L.; Denti, G.; Fanelli, G. Estimation of growth parameters of the black scallop *Mimachlamys varia* in the gulf of Taranto (Ionian Sea, Southern Italy). *Water* **2020**, *12*, 3342. [CrossRef]
32. Strand, Ø.; Louro, A.; Duncan, P.F. European aquaculture. In Developments in Aquaculture and Fisheries Science. *Elsevier* **2016**, *40*, 859–890.
33. Kovačić, I.; Burić, P.; Žunec, A.; Bilić, J.; Prgić, A.; Čanak, I.; Iveša, N.; Štifanić, M.; Frece, J. The Effect of *Lactiplantibacillus plantarum* I-Enriched Diet on the Phenolic Content and Antioxidant Capacity of Queen Scallop (*Aequipecten opercularis* Linnaeus, 1758) Extracts. *Microorganisms* **2023**, *11*, 2723. [CrossRef]
34. Kovačić, I.; Žunec, A.; Matešković, M.; Burić, P.; Iveša, N.; Štifanić, M. Seasonal changes in morphometric and physiological parameters of the queen scallop *Aequipecten opercularis* (Linnaeus, 1758) cultured in captivity. In *Croatian Biology Congress Book of Abstracts*; Croatian Scientific Bibliography: Pula, Croatia, 2022; Available online: https://www.bib.irb.hr:8443/1225620 (accessed on 24 April 2024).
35. Cataldo, D.H.; Boltovskoy, D.; Stripeikis, J.; Pose, M. Condition index and growth rates of field caged *Corbicula fluminea* (Bivalvia) as biomarkers of pollution gradients in the Paraná river delta (Argentina). *Aquat. Ecosyst. Health Manag.* **2001**, *4*, 187–201. [CrossRef]
36. Blaise, C.; Gagné, F.; Burgeot, T. Three simple biomarkers useful in conducting water quality assessments with bivalve mollusks. *Environ. Sci. Pollut. Res.* **2017**, *24*, 27662–27669. [CrossRef]
37. Johnson, A.L.A.; Hickson, J.A.; Swan, J.; Brown, M.R.; Heaton, T.H.E.; Chenery, S.; Balson, P.S. The Queen Scallop *Aequipecten opercularis*: A new source of information on late Cenozoic marine environments in Europe. *Geol. Soc. Lond. Spec. Publ.* **2000**, *177*, 425–439. [CrossRef]
38. Fernández-Reiriz, M.J.; Range, P.; Álvarez-Salgado, X.A.; Espinosa, J.; Labarta, U. Tolerance of juvenile *Mytilus galloprovincialis* to experimental seawater acidification. *Mar. Ecol. Prog. Ser.* **2012**, *454*, 65–74. [CrossRef]
39. Mackenzie, C.L.; Ormondroyd, G.A.; Curling, S.F.; Ball, R.J.; Whiteley, N.M.; Malham, S.K. Ocean warming, more than acidification, reduces shell strength in a commercial shellfish species during food limitation. *PLoS ONE* **2014**, *9*, e86764. [CrossRef]
40. Ong, E.Z.; Briffa, M.; Moens, T.; Van Colen, C. Physiological responses to ocean acidification and warming synergistically reduce condition of the common cockle *Cerastoderma edule*. *Mar. Environ. Res.* **2017**, *130*, 38–47. [CrossRef]
41. Byrne, M.; Foo, S.A.; Ross, P.M.; Putnam, H.M. Limitations of cross- and ultigenerational plasticity for marine invertebrates faced with global climate change. *Glob. Chang. Biol.* **2020**, *26*, 80–102. [CrossRef]
42. Kamermans, P.; Saurel, C. Interacting climate change effects on mussels (*Mytilus edulis* and *M. galloprovincialis*) and oysters (*Crassostrea gigas* and *Ostrea edulis*): Experiments for bivalve individual growth models. *Aquat. Living Resour.* **2022**, *35*, 1. [CrossRef]
43. Schmidt, M.; Philipp, E.E.; Abele, D. Size and age-dependent changes of escape response to predator attack in the Queen scallop *Aequipecten opercularis*. *Mar. Biol. Res.* **2008**, *4*, 442–450. [CrossRef]
44. Çolakoğlu, S.; Çolakoğlu, F.; Künili, İ.E. Length–Weight Relationships, Meat Yield and Morphometric Indices of Five Commercial Bivalve Species Collected from the Çanakkale Strait (Türkiye). *Aquat. Sci. Eng.* **2023**, *39*, 36–42. [CrossRef]
45. Vereycken, J.E.; Aldridge, D.C. Bivalve molluscs as biosensors of water quality: State of the art and future directions. *Hydrobiologia* **2022**, *850*, 231–256. [CrossRef]
46. Gazeau, F.; Alliouane, S.; Bock, C.; Bramanti, L.; López, C.M.; Gentile, M.; Hirse, T.; Pörtner, H.; Ziveri, P. Impact of ocean acidification and warming on the Mediterranean mussel (*Mytilus galloprovincialis*). *Front. Mar. Sci.* **2014**, *1*, 62–74. [CrossRef]
47. Watson, S.; Southgate, P.C.; Miller, G.M.; Moorhead, J.A.; Knauer, J. Ocean acidification and warming reduce juvenile survival of the fluted giant clam, *Tridacna squamosa*. *Molluscan Res.* **2012**, *32*, 177–180. [CrossRef]
48. Gooding, R.A.; Harley, C.D.G.; Tang, E. Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 9316–9321. [CrossRef] [PubMed]
49. Adedeji, O.; Reuben, O.; Olatoye, O. Global climate change. *J. Geosci. Environ. Prot.* **2014**, *2*, 114. [CrossRef]

50. Dash, G.; Raman, R.P.; Prasad, K.P.; Makesh, M.; Pradeep, M.A.; Sen, S. Evaluation of *Lactobacillus plantarum* as feed supplement on host associated microflora, growth, feed efficiency, carcass biochemical composition and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquaculture* **2014**, *432*, 225–236. [CrossRef]
51. Dash, G.; Raman, R.P.; Prasad, K.P.; Makesh, M.; Pradeep, M.A.; Sen, S. Evaluation of paraprobiotic applicability of *Lactobacillus plantarum* in improving the immune response and disease protection in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Fish Shellfish Immun.* **2015**, *43*, 167–174. [CrossRef] [PubMed]
52. Dash, G.; Raman, R.P.; Prasad, K.P.; Marappan, M.; Pradeep, M.A.; Sen, S. Evaluation of *Lactobacillus plantarum* as a water additive on host associated microflora, growth, feed efficiency and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquac. Res.* **2016**, *47*, 804–818. [CrossRef]
53. Ringø, E. Probiotics in shellfish aquaculture. *Aquac. Fish.* **2020**, *5*, 1–27. [CrossRef]
54. Sun, F.; Wang, Y.; Wang, C.; Zhang, L.; Tu, K.; Zheng, Z. Insights into the intestinal microbiota of several aquatic organisms and association with the surrounding environment. *Aquaculture* **2019**, *507*, 196–202. [CrossRef]
55. Vasconcelos, P.; Moura, P.; Pereira, F.; Pereira, A.M.; Gaspar, M.B. Morphometric relationships and relative growth of 20 uncommon bivalve species from the Algarve coast (southern Portugal). *J. Mar. Biol. Assoc. U. K.* **2018**, *98*, 463–474. [CrossRef]
56. Caill-Milly, N.; Bru, N.; Mahé, K.; Borie, C.; d'Amico, F. Shell shape analysis and spatial allometry patterns of Manila clam (*Ruditapes philippinarum*) in a mesotidal coastal lagoon. *J. Mar. Biol.* **2012**, *2012*, 281206. [CrossRef]
57. Caill-Milly, N.; Bru, N.; Barranger, M.; Gallon, L.; D'amico, F. Morphological trends of four Manila clam populations (*Venerupis philippinarum*) on the French Atlantic coast: Identified spatial patterns and their relationship to environmental variability. *J. Shellfish Res.* **2014**, *33*, 355–372. [CrossRef]
58. Ricker, W.E. Linear regressions in fishery research. *J. Fish. Board Can.* **1973**, *30*, 409–434. [CrossRef]
59. Gaspar, M.B.; Santos, M.N.; Vasconcelos, P.; Monteiro, C.C. Shell morphometric relationships of the most common bivalve species (Mollusca: Bivalvia) of the Algarve coast (southern Portugal). *Hydrobiologia* **2002**, *477*, 73–80. [CrossRef]
60. Huxley, J.S.; Teissier, G. Terminology of relative growth. *Nature* **1936**, *137*, 780–781. [CrossRef]
61. Nascimento-Schulze, J.C.; Bean, T.P.; Houston, R.D.; Santos, E.M.; Sanders, M.B.; Lewis, C.; Ellis, R.P. Optimizing hatchery practices for genetic improvement of marine bivalves. *Rev. Aquac.* **2021**, *13*, 2289–2304. [CrossRef]
62. Smaal, A.C.; Ferreira, J.G.; Grant, J.; Petersen, J.K.; Strand, Ø. *Goods and Services of Marine Bivalves*; Springer Nature: Berlin, Germany, 2019; Volume 591. [CrossRef]
63. Tan, K.; Zhang, H.; Zheng, H. Selective breeding of edible bivalves and its implication of global climate change. *Rev. Aquac.* **2020**, *12*, 2559–2572. [CrossRef]
64. Pagano, G.; Castello, G.; Gallo, M.; Borriello, I.; Guida, M. Complex Mixture-Associated Hormesis and Toxicity: The Case of Leather Tanning Industry. *Dose-Response* **2008**, *6*, 383–396. [CrossRef]
65. Range, P.; Piló, D.; Ben-Hamadou, R.; Chícharo, M.A.; Matias, D.; Joaquim, S.; Oliveira, A.P.; Chícharo, L. Seawater acidification by CO_2 in a coastal lagoon environment: Effects on life history traits of juvenile mussels *Mytilus galloprovincialis*. *J. Exp. Mar. Biol. Ecol.* **2012**, *424*, 89–98. [CrossRef]
66. Ozvarol, Y.; Gokoglu, M. Some Biological Aspects of Scallop *Chlamys varia* (Linnaeus, 1758), Bivalvia: Pectinida from Aegean Sea coast of Turkey. *J. Appl. Biol. Sci.* **2013**, *7*, 68–70.
67. Tate, R.D.; Benkendorff, K.; Ab Lah, R.; Kelaher, B.P. Ocean acidification and warming impacts the nutritional properties of the predatory whelk, *Dicathais orbita*. *J. Exp. Mar. Biol. Ecol.* **2017**, *493*, 7–13. [CrossRef]
68. Enríquez-Díaz, M.; Pouvreau, S.; Chávez-Villalba, J.; Le Pennec, M. Gametogenesis, reproductive investment, and spawning behavior of the Pacific giant oyster *Crassostrea gigas*: Evidence of an environment-dependent strategy. *Aquac. Int.* **2009**, *17*, 491–506. [CrossRef]
69. Sreedevi, P.R.; Uthayakumar, V.; Jayakumar, R.; Ramasubramanian, V. Influence of rearing water temperature on induced gonadal development and spawning behaviour of tropical green mussel, *Perna viridis*. *Asian Pac. J. Reprod.* **2014**, *3*, 204–209. [CrossRef]
70. Suja, N.; Muthiah, P. Effect of starvation and temperature on gonad development of baby clam, *Marcia opima* (Gmelin). *J. Mar. Biol. Assoc. India* **2009**, *51*, 21–25.
71. Delgado, M.; Pérez-Camacho, A. Comparative study of gonadal development of *Ruditapes philippinarum* (Adams and Reeve) and *Ruditapes decussatus* (L.) (Mollusca: Bivalvia): Influence of temperature. *Sci. Mar.* **2007**, *71*, 471–484. [CrossRef]
72. Beltrán-Lugo, A.I.; Maeda-Martínez, A.N.; Pacheco-Aguilar, R.; Nolasco-Soria, H.G. Seasonal variations in chemical, physical, textural, and microstructural properties of adductor muscles of Pacific lions-paw scallop (*Nodipecten subnodosus*). *Aquaculture* **2006**, *258*, 619–632. [CrossRef]
73. Pichaud, N.; Briatte, S.; Desrosiers, V.; Pellerin, J.; Fournier, M.; Blier, P.U. Metabolic capacities and immunocompetence of sea scallops (*Placopecten magellanicus*, Gmelin) at different ages and life stages. *J. Shellfish Res.* **2009**, *28*, 865–876. [CrossRef]
74. Pilditch, C.A.; Grant, J. Effect of temperature fluctuations and food supply on the growth and metabolism of juvenile sea scallops (*Placopecten magellanicus*). *Mar. Biol.* **1999**, *134*, 235–248. [CrossRef]