fishes

MDPI

Article

Environmentally Friendly and Efficient Methods for Mitigating the Density of Ascidian Fouling in Mediterranean Mussel Farming

Dimitrios Tsotsios [1], Dimitrios K. Papadopoulos [2], Maria V. Alvanou [3], Ioannis Georgoulis [2], Athanasios Lattos [2], Basile Michaelidis [2], Konstantinos Feidantsis [1], Ioannis A. Giantsis [3,*] and John A. Theodorou [1,*]

[1] Department of Fisheries and Aquaculture, University of Patras, 30200 Mesolongi, Greece; kfeidant@upatras.gr (K.F.)
[2] Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[3] Laboratory of Ichthyology and Fisheries, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
* Correspondence: igiants@agro.auth.gr (I.A.G.); jtheo@upatras.gr (J.A.T.)

Abstract: Ascidian biofouling generates significant challenges to bivalve aquaculture. Their rapid spread across almost every available surface leads to increased maintenance costs and reduced yields in shellfish farming. In addition, ascidians may introduce pathogens or toxins, further compromising the health and marketability of bivalve stocks and thereby necessitating strict management strategies to manage these impacts. The aim of this study was the evaluation of different management practices for eliminating ascidian fouling and the identification of the best method for *Mytilus galloprovincialis* (Lamarck, 1819) aquaculture farms. The effects of different anti-fouling treatments as well as their interactive outcomes were examined by conducting two experiments. Various experimental procedures were applied, including the temporally differential washing of mussels, air exposure and immersion in 50 ppt and 70 ppt salinity solutions, as well as the combination of these applications. All treatments reduced the number of ascidian colonies on mussel socks but at varying proportions. Immersing mussel socks in a 70 ppt salinity solution followed by air exposure for 1 day was the most efficient method and led to a 93% eradication.

Keywords: biofouling; tunicates; aquaculture; *Mytilus galloprovincialis*; bivalves; management practices

Key Contribution: Biofouling constitutes a severe problem in mussel culture, not only for the equipment, as in other aquaculture forms, but also for the reared mussels, which represent an ideal substrate for fouling organisms. We evaluated different combinations of anti-fouling treatments and propose that a short immersion of fouled cultured mussels in a 70 ppt salinity solution followed by a 24 h air exposure provides the most promising results. This method is completely eco-friendly, avoiding chemicals or other repellents.

check for updates

Citation: Tsotsios, D.; Papadopoulos, D.K.; Alvanou, M.V.; Georgoulis, I.; Lattos, A.; Michaelidis, B.; Feidantsis, K.; Giantsis, I.A.; Theodorou, J.A. Environmentally Friendly and Efficient Methods for Mitigating the Density of Ascidian Fouling in Mediterranean Mussel Farming. *Fishes* **2024**, *9*, 135. https://doi.org/10.3390/fishes9040135

Academic Editor: Amit Kumar Sinha

Received: 19 March 2024
Revised: 9 April 2024
Accepted: 12 April 2024
Published: 14 April 2024

1. Introduction

Biofouling poses a significant threat to mussel aquaculture, as it leads to direct economic losses. The proliferation of this phenomenon and its impacts on aquaculture have led to the need to research the economic impacts on bivalve farming [1]. In the European aquaculture industry alone, costs were estimated to range from 5% to 10% of the industry's total value [2]. Previous assessments considering the final market prices have indicated that biofouling by various fouling organisms could cause economic losses of up to 20% for oyster farming [3] and 30% for scallop aquaculture [4]. The increased weight of the

fouled infrastructure also raises the production costs since it involves multiple actions for cleaning [5].

Ascidians are among the most persistent sources of biofouling in shellfish farming worldwide and can reach very high density or biomass in a relatively short time; for example, *Ciona intestinalis* and *Styela plicata* can establish population numbers up to 10^4 fouling individuals per hectare [6–10]. These organisms compete with mussels for the availability of food and oxygen and also for space on the substrate (net) [6,7], sometimes leading to a total loss of the mussel population as they detach and fall to the bottom of the sea [6,7]. The effects of ascidian fouling include increased costs of production up to 50% [11,12] and increased processing, negative effects on the growth rates of the cultured species and decreased quality of the final product [13]. These animals threaten shellfish farming sustainability since they may represent up to 30% of the total operating costs in a shellfish farm [14]. Thus, understanding their biological characteristics is of particular importance, and the mitigation of such impacts is crucial for the development of effective management strategies [15].

The periodicity of ascidian reproduction varies widely [16] and is poorly known for many species [17], but it is more than clear that water temperature is a dominant factor [18,19]. For temperate ascidians, spawning usually occurs during the summer months [20], with a subsequent decline during cooler months. In contrast, ascidian populations in warmer waters release gametes continuously throughout the year [14,21].

Avoidance and prevention are the first-line responses, but when surviving populations reach harmful densities, they require immediate treatment [22]. Several biofouling management methods have been developed for shellfish farming, including physical [23], chemical [24], biological [25] and mechanical methods [26–28]. Despite the great extent of existing work available, commonly applied approaches are limited. A meta-analysis of biofouling treatment methods in shellfish farming revealed that the applied approaches generally have neutral or negative effects on cultured animals [29]. This finding reflects the lack of information and specific criteria about when and how to apply each method (treatment) for net-positive results. Furthermore, when introducing laboratory-scale evaluations in a culture farm, significant operational challenges are met regarding the development of applicable protocols and reliable quality control measurements [30]. In situ treatment methodologies, combined with efficient and low-cost mitigation strategies, are essential, preferably along with little environmental impact and minimal effects on the farmed species.

The goal of the present study was the comparison of eco-friendly mitigation methods for the elimination of ascidians in terms of efficiency. Field experiments were conducted in aquaculture farms of the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819) and focused on the ascidian eradication efficiency of each treatment on the mussel socks of the farm. Washing, air exposure, high-salinity immersion and combinations of these methods were explored, and ascidian numbers on the socks were recorded for each treatment.

2. Materials and Methods

2.1. Study Area Description

The field experiments were implemented on the installations of two mussel farms, one longline and one raft mussel farm on the outer side of the estuary/gate to the Mazoma Lagoon, which is located on the northwest coast of Amvrakikos Gulf, next to the city of Preveza, Epirus (NW Greece) (Figure 1). Amvrakikos Gulf resembles a fjord with an area of 525 km^2. The renewal of its waters takes place from the Ionian Sea through the long but shallow "mouth" of the Gulf (Preveza–Aktio channel). The water quantity entering the gulf is insufficient to renew the entire water mass, which is supplemented by the rivers Arachthos and Louros. Louros (length 80 km, average annual discharge 400×103 m^3 $year^{-1}$); this provides high amounts of nutrients to the gulf, thus supporting intensive agricultural activity and livestock production [31–34]. The hydrological profile of nutrient fall in combination with the limited renewal of sea water masses leads to vertical

stratification at 6–10 m, which abruptly changes the environmental conditions to hypoxic (oxygen concentrations < 2 mg L^{-1}) or anoxic (oxygen concentrations < 0.2 mg L^{-1}) near the river bottom [35,36]. The abundance of zooplankton including larval bivalves declines with increasing depth. A uniform vertical distribution is presented due to the mixing of water late in autumn (October–November) [33,37,38].

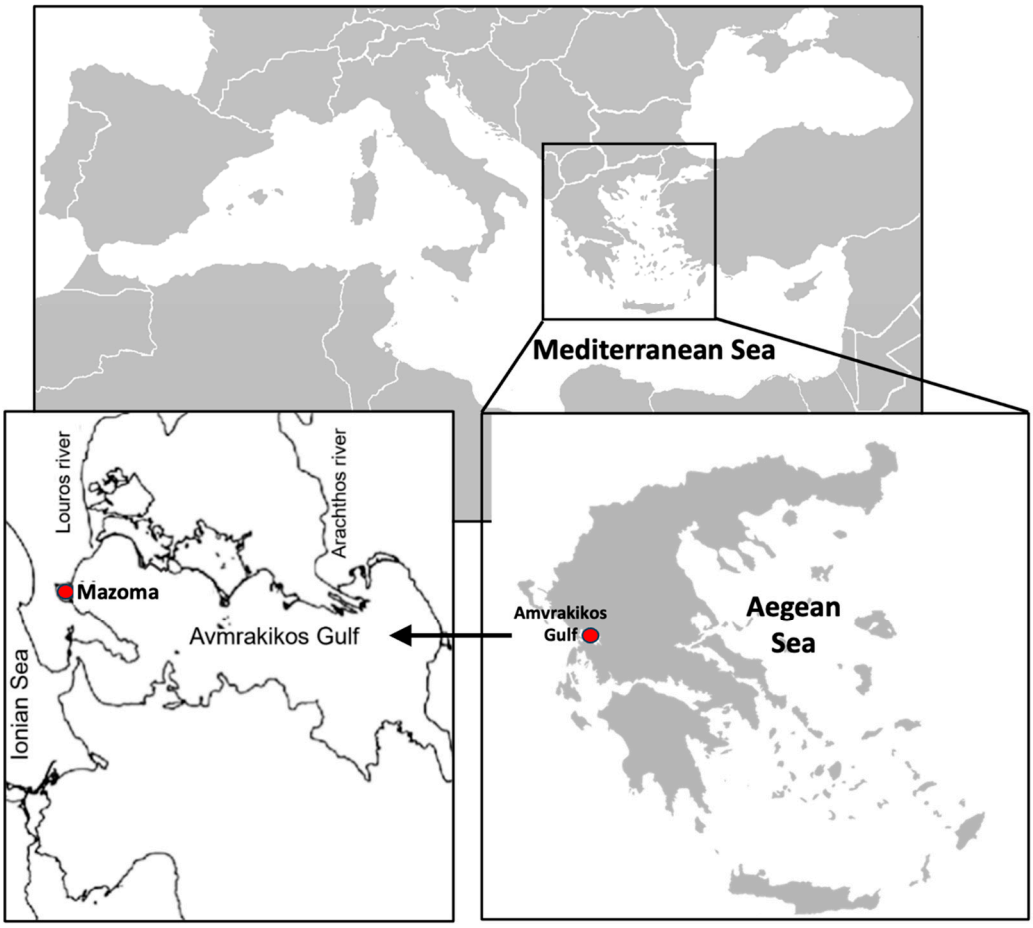

Figure 1. Map location of the Amvrakikos Gulf and the area of Mazoma Lagoon where the mussel farm in which the field experimental procedure was conducted is located. Coordinates: 39.025628318910435, 20.7571601812546.

2.2. Preparation of Mussel Socks

In mid-June 2021, 80 mussel socks were filled with mussel spat of mean length 2.2 cm. They were cleaned as much as possible to be free of ascidians or any other epifaunal organisms by washing them with sea water prior to their placement on a raft in the shellfish farm. The mussel socks were constructed from plastic culture nets, 3 m long each, with a culture density of 100 mussels per 10 cm sock length.

2.3. Field Experiment (Mussel Sock Washing Duration and/or Air Exposure): Designation of Experiment 1

Four treatments were employed from 15 June 2022 to 15 November 2022 (150-day trial). A total of 20 mussel socks were divided into 5 groups of 4 socks each: (a) group A was washed with sea water of 35.3 psu salinity every 15 days; (b) group B was washed with sea water every 30 days; (c) group C was exposed to the air in a continuously shaded place under a canopy for 24 h every 30 days and then returned to the sea; (d) group D was washed every 30 days, subsequently exposed to air for 24 h, and the next day it was again placed in the farm; and e) a control group that was not subjected to any treatment (Table 1, Figures 2 and 3).

For the mussel sock washing, a water pump (170 L min^{-1} capacity 2″ hose section, U = 2.5 ms^{-1}) was used, which provided enough water to wash and clean the mussel socks while avoiding mussel mortality and losses owing to potential falls to the bottom of the sea [25–27]. The mussel socks were transferred from the raft to the boat, mussel mortality was recorded by visual observations, and the ascidian colonies were counted. In each sock, three parts of 30 cm each [one at the top (0 m), one in the middle (1.5 m) and one at the lower end (3 m) were defined, and the ascidian colonies was counted. All data were quadrupled by collecting four mussel samples from each depth. In addition, temperature and dissolved oxygen (DO) were measured every month in the field using a Handy Polaris Oxyguard oxygen meter since they represent important abiotic factors affecting ascidian population development [6,7], while sea water samples were sent monthly to the University of Patras for determination of the concentration of Chl-α. Salinity is relatively stable in the study area, ranging between approximately 35 and 36 psu, and hence it was not periodically measured. The biological cycles of ascidians are season-dependent, and their abundance and reproduction peak occur during the warmest months. The settlement period is short, usually taking place during late summer and early autumn [12,18]. Sea water temperatures seem to play a vital role in the development of ascidian colonies on the mussel socks.

Table 1. Overview of experiment 1.

Group	Method	Period	Duration
Group a	Sea water washing	Every 15 days	1 July–31 October
Group b	Sea water washing	Every 30 days	1 July–31 October
Group c	Air exposure	Every 30 days	1 July–31 October
Group d	Sea water washing and air exposure	Every 30 days	1 July–31 October
Group e	Control		

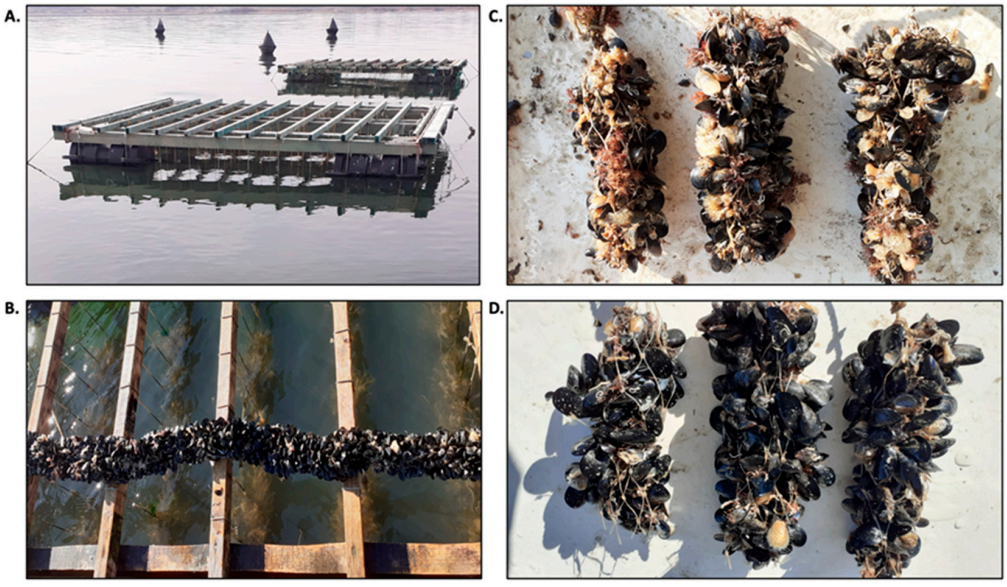

Figure 2. Longline and raft mussel farm installation located close to the Mazoma Lagoon (northwest coast of Amvrakikos Gulf) (**A**); where mussel socks were placed (**B**). Mussel socks before (**C**) and after (**D**) the washing with sea water and/or air exposure; (**C**,**D**) were obtained after transferring the mussels to a sunny place in order for them to be better displayed.

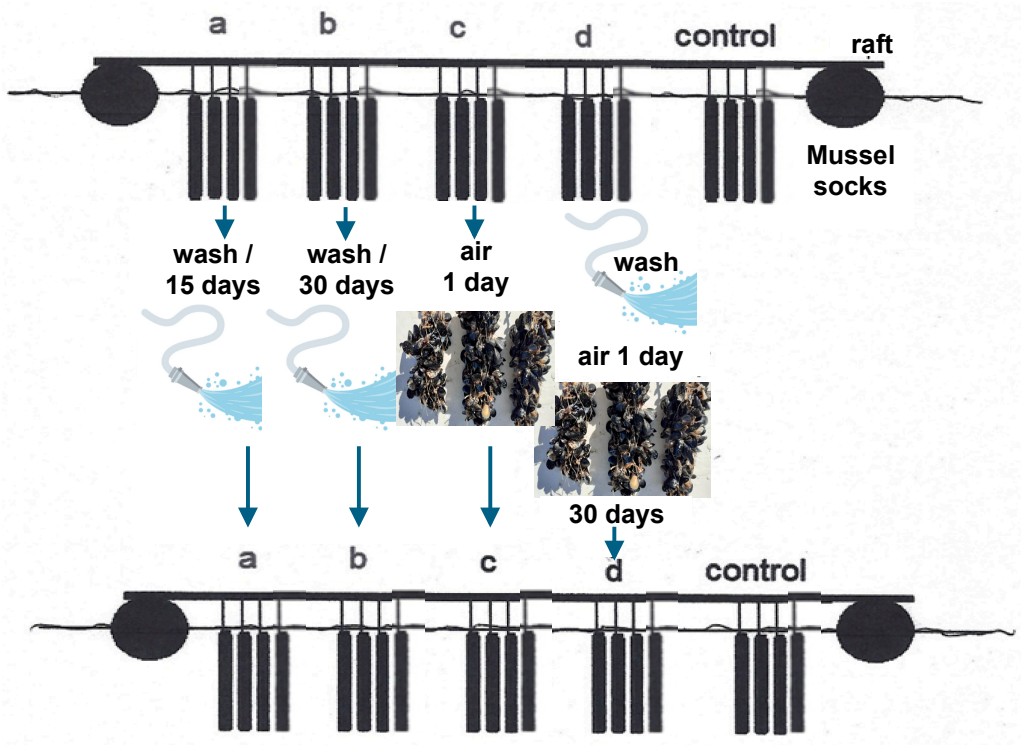

Figure 3. Schematic representation of experiment 1 in the four different groups of mussel socks (a–d), as described in the Section 2.3.

2.4. Laboratory Experiment (Immersion of Mussel Socks in High-Salinity Solutions): Designation of Experiment 2

At the beginning of October 2022, as the ascidian colonies were well developed, untreated mussel socks from the same raft and longlines of the mussels in experiment 1 were employed for the laboratory experiment. Mussel sock pieces were immersed in high-salinity solutions. Socks were cut into 25 cm length pieces, forming 9 groups of 4 pieces (total of 36 mussel sock pieces of 25 cm each). On all sock pieces, ascidian colonies were counted, while one group of four mussel socks pieces was employed as the control. For the mussels' immersion, three 50-liter tanks filled with sea water and salt were added to increase to the desired salinity. A hand salinometer was used to register the water salinity. Two groups (groups 50/3 and 50/5) were immersed in a 50 ppt salinity solution for 3 and 5 min. Two other groups (groups 70/3 and 70/5) were immersed in a 70 ppt salinity solution for 3 and 5 min, respectively. After their immersion, the mussel sock pieces of groups 50 and 70 were returned to the sea at the farming area. The next four groups were immersed in 50 ppt (groups 50/3a and 50/5a) and 70 ppt (groups 70/3a and 70/5a) salinity solutions for 3 and 5 min, respectively, for each group, followed by exposure to air for 24 h under shadow (Table 2, Figures 4 and 5). The next day, they were re-immersed in the sea by hanging them from the raft. After an 8-day period, the mussel sock pieces were harvested, and the ascidian colonies were counted.

Table 2. Overview of experiment 2.

Group	Immersion	Duration	Air exposure	Duration
50/3	50 ppt salinity solution	3 min		8 days
50/5	50 ppt salinity solution	5 min		8 days
70/3	70 ppt salinity solution	3 min		8 days
70/5	70 ppt salinity solution	5 min		8 days
50/3a	50 ppt salinity solution	3 min	24 h	8 days
50/5a	50 ppt salinity solution	5 min	24 h	8 days
70/3a	70 ppt salinity solution	3 min	24 h	8 days
70/5a	70 ppt salinity solution	5 min	24 h	8 days
control				8 days

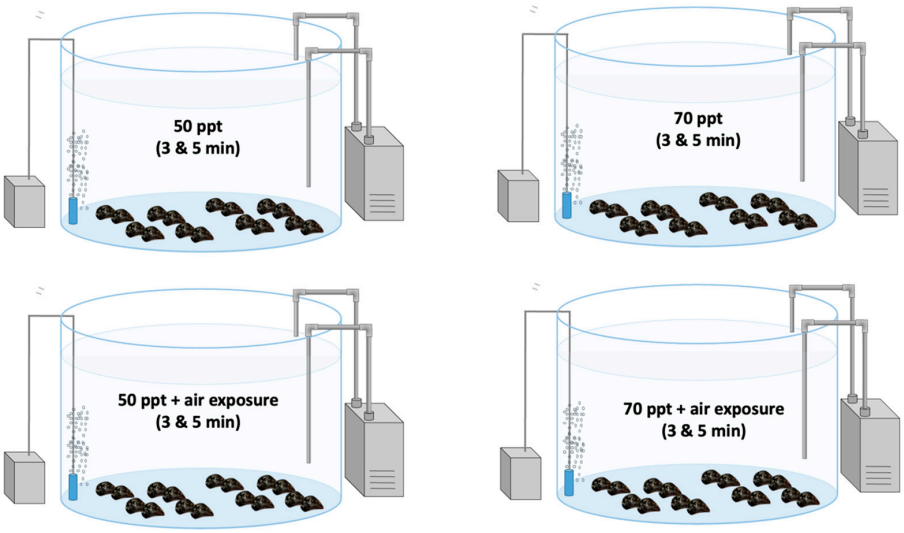

Figure 4. Schematic representation of the experimental protocol employing sea water tanks of different salinities and different immersion times of mussels.

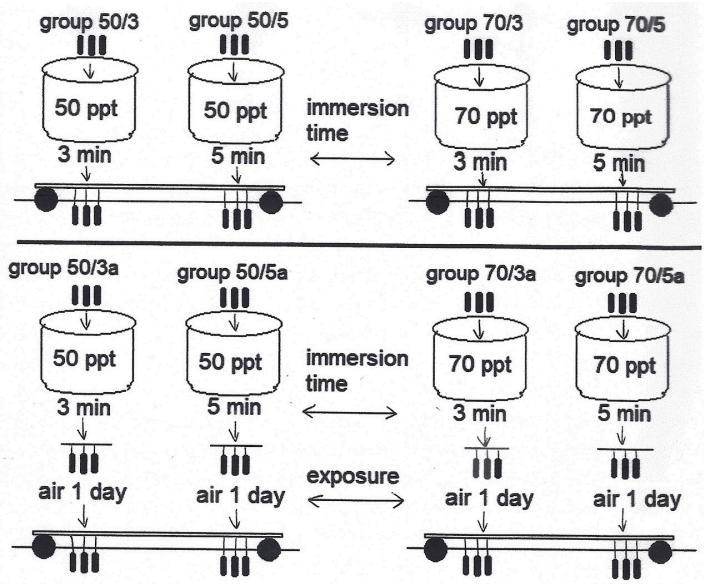

Figure 5. Schematic representation of experiment 2.

2.5. Data Treatment and Statistical Analysis

The statistical analysis of the results was performed using SPSS 22.0. Comparisons among the recorded values of mortality and ascidian densities were performed by one-way analysis of variance (ANOVA), attributing significance to a 5% confidence level ($p < 0.05$). The Bonferroni test, followed by Dunn's post-test, was employed to perform post hoc comparisons.

3. Results

3.1. Sea Water Physicochemical Parameters

The sea water temperature started increasing in May and reached its highest levels, i.e., 29.7 °C, in July, with its levels decreasing again in September and reaching its lowest levels in December, i.e., 12.8 °C. While the dissolved oxygen concentration remained stable throughout the year at approximately 9–10 mg/L, the Chl-α concentration level started increasing in July at 4.1 mg/L, reached its maximum level in August at 4.7 mg/L and remained high until December (Figure 6).

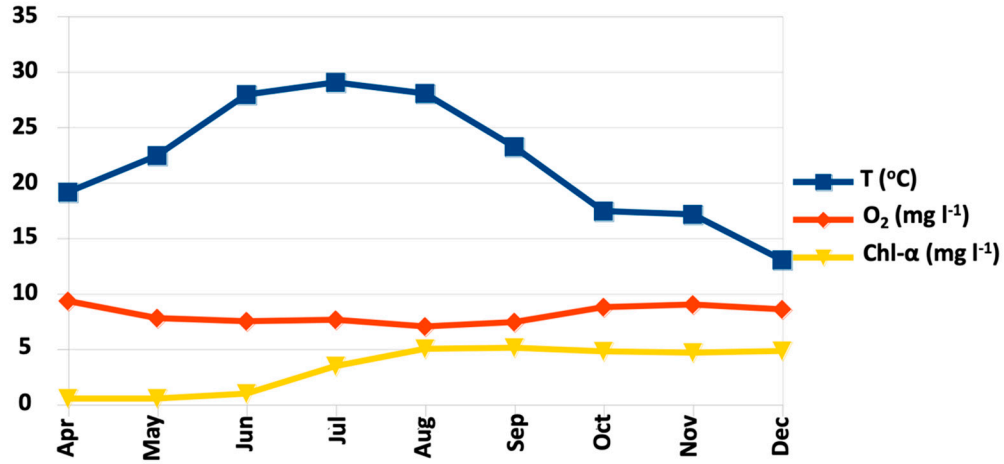

Figure 6. Seasonal variations in sea water temperature, oxygen and Chl-α concentration in the Mazoma Lagoon mussel farm.

3.2. Mussel Sock Washing and/or Air Exposure

As demonstrated in the control group (Figure 7), there was a heavy ascidian recruitment rate at the beginning of the experimental period (July and August), followed by a stabilization over the next months, whereas on the other hand, a less than 2% mortality of mussels was observed. Washing the mussel socks every 15 days resulted in a decline of the already attached ascidian colonies on the socks in August and September. Washing with sea water every 15 days resulted in a gradually decreasing number of ascidian colonies on the mussel socks, with the lowest values observed on the 15th of November. On the other hand, control mussel socks exhibited a gradual increase in the number of colonies. Statistically significant differences between control and washed mussel socks were already observed after the first 15 days of washing on the 15th of July (Figure 7).

The same pattern was also observed when mussel socks were treated every 30 days either by washing, air exposure or by a combination of washing and air exposure. Exposing the mussel socks to air in a shaded area for 24 h significantly decreased the ascidian recruitment. Compared to the control group at the end of the experimental period, only 19.6% of the colonies remained on the socks exposed to air (Figure 8B), while 4% of the colonies remained on the washed and air-exposed socks (Figure 8C). While control mussel socks exhibited increased ascidian colonies, washing, air exposure and the synergy of washing and air exposure resulted in gradual and significant decreases (Figure 8A–C). Finally, a comparison of these three treatments (Figure 8D) showed that the synergy of

washing and air exposure was more efficient in ascidian removal from the mussel socks, followed by washing and lastly by air exposure.

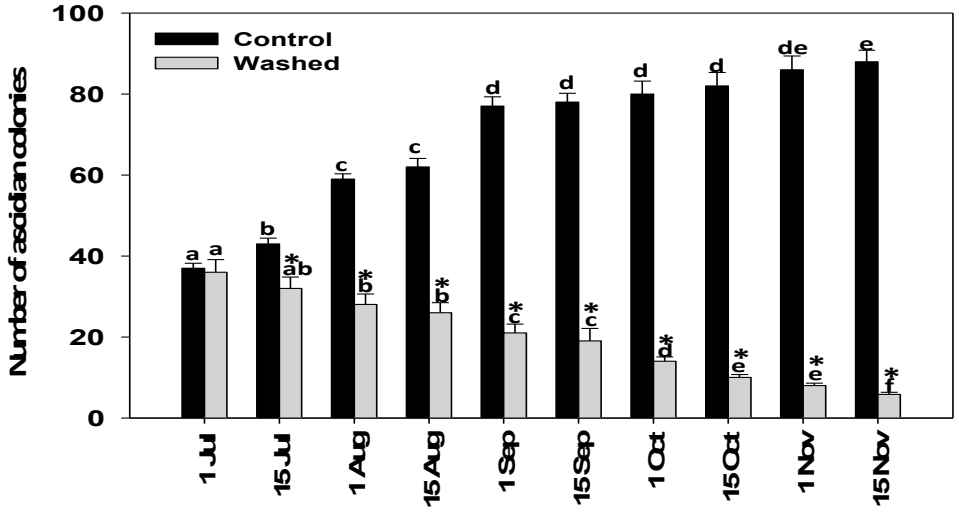

Figure 7. Fifteen-day washing effect for a period of 5 months on the number of ascidian colonies of the mussel socks. Values are mean ± S.D. of $n = 4$. Lower case letters denote statistically significant differences ($p < 0.05$) between different time periods of intervention, while asterisks (*) denote statistically significant differences ($p < 0.05$) between control and treated mussels.

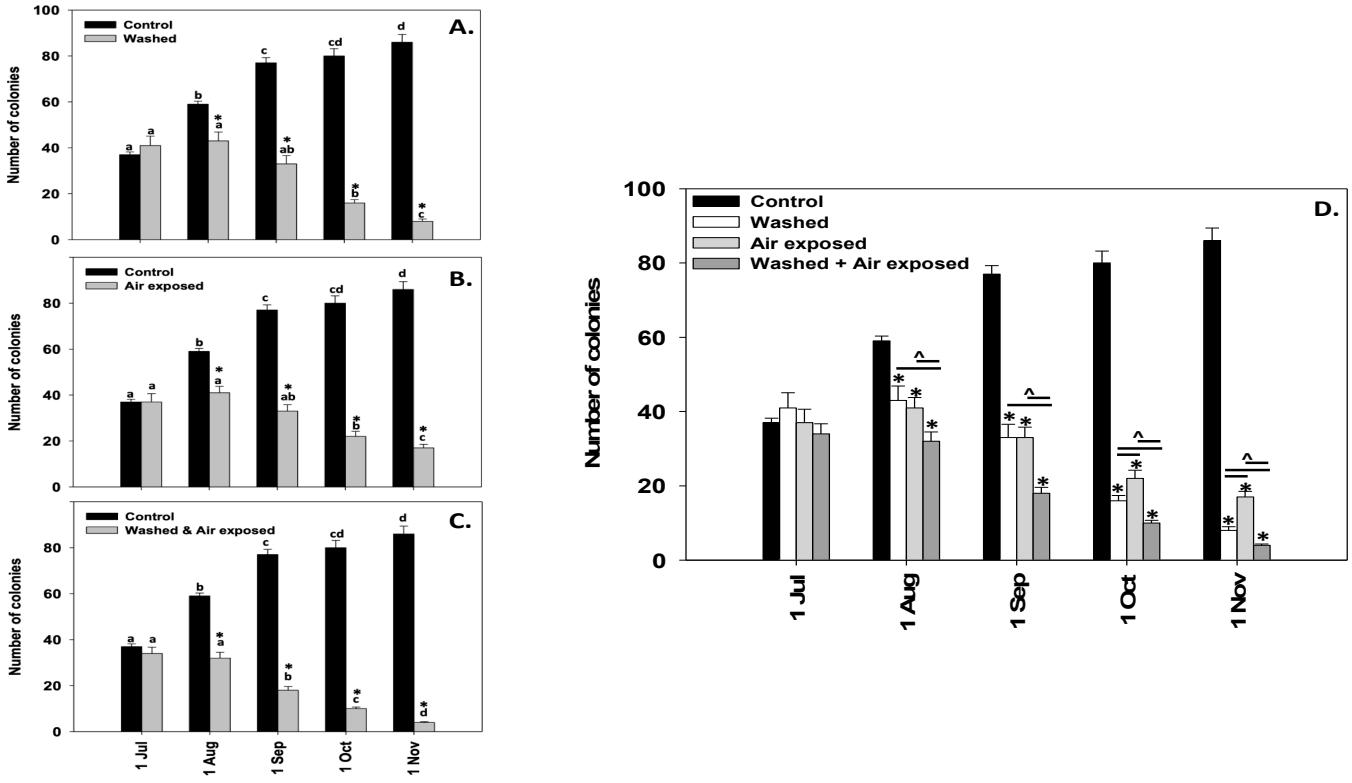

Figure 8. Thirty-day washing (**A**), air exposure (**B**), combination of washing and air exposure effect (**C**) and comparison between all treatments (**D**) for a period of five months on the number of ascidian colonies of the mussel socks. Values are mean ± S.D. of $n = 4$. Lower case letters denote statistically significant differences ($p < 0.05$) between different time periods of intervention, asterisks (*) denote statistically significant differences ($p < 0.05$) between control and treated mussel socks, and carets (^) denote statistically significant differences ($p < 0.05$) between different treatments.

3.3. Immersion of Mussel Socks in High-Salinity Solutions

No mussel mortality occurred during the treatment period. The immersion of mussels into sea water of 50 and 70 ppt salinity resulted in no changes in the number and the % maintenance of ascidian colonies. Mortality ranged from 8% to 9% in the 50 ppt salinity bath for 3 min and 5 min, respectively, while for the 70 ppt solution the mortality ranged from 10% to 11%. However, immersion in the 50 ppt salinity sea water and air exposure resulted in a decreased number and the decreased % maintenance of ascidian colonies after the mussels were treated. Immersing mussel socks in 50 ppt salinity and then exposing them to air in a shaded place for 24 h yielded an ascidian mortality of 22% in the 3 min period and 32% in the 5 min period. On the other hand, immersion in the 70 ppt salinity sea water for 3 or 5 min and air exposure decreased the number and the % maintenance of ascidian colonies, resulting in ascidian mortality rates of 86% and 93%, respectively. It should be underlined that in both of the aforementioned treatments, the final number of colonies was statistically decreased compared to the initial number; however, the 5 min duration was more effective than the 3 min one (Figure 9).

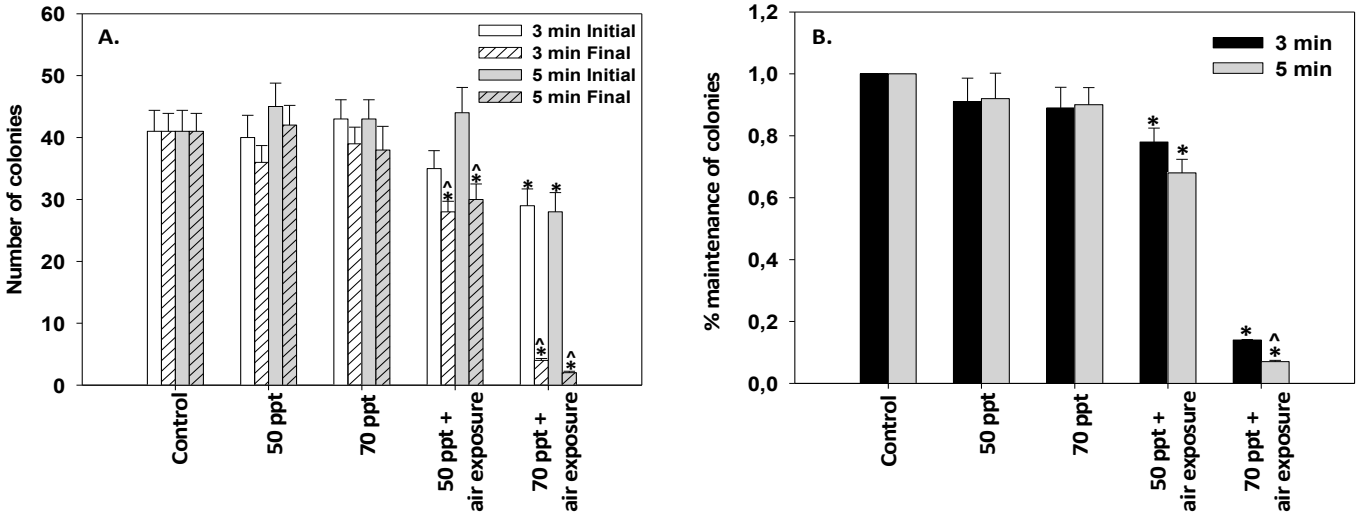

Figure 9. Effect of immersion in 50 and 70 ppt sea water for 3 and 5 min and exposure to air on the number (**A**) and the (**B**) % maintenance of ascidian colonies on the mussel socks. Values are mean ± S.D. of $n = 4$. Asterisks (*) denote statistically significant differences ($p < 0.05$) between control and treated mussel socks and carets (^) denote statistically significant differences ($p < 0.05$) before and after treatment (**A**) and between different time exposures (3 and 5 min) (**B**).

4. Discussion

4.1. Comparison of the Different Treatments

In our study, ascidian presence varied seasonally, increasing during the summer and declining in the autumn, in accordance with the reduced water temperatures. With reference to the first experimental sock washing, exposure to air and the combination of washing and exposure to air reduced the ascidian colonies at different levels. The continuously high number of ascidians even after their removal is likely the result of re-colonization of the treated mussel socks over time [39], either through the budding of surviving zooids or through post-settlement of free-swimming larvae [25]. The low-pressure washing method had no adverse effects on mussels since no mussel mortality was observed on the mussel socks during the experimental period. Compared to the control, washing the mussel socks every 15 and 30 days reduced the ascidian colonies on socks by up to 9.6 times. Paetzold et al. [27], using high-pressure washing, recorded a 30% loss of mussels. Arens et al. [26] suggested that high-pressure washing significantly reduced *Botryllus schlosseri* biomass, but treatment timing rather than frequency was the most important factor. The 15-day or the 30-day application of sea water washing in this study provided satisfying results because

the frequent washing possibly detached the newly recruited larvae that were in early stages of development and were more sensitive to the treatment.

The combination of washing and exposing the mussel socks to the air every 30 days yielded the greatest reduction in ascidians in the first experimental series. The lowest reduction was observed in the group exposed only to air every 30 days. It should be pointed out that the initial washing of the mussel socks and the removal of competing epifauna before rehanging them in the raft was crucial, possibly because these organisms provided a more suitable substrate on the mussel sock with multiple microhabitats for settlement and proliferation of the ascidians. At the beginning of the experiment, the washing and air exposure techniques were partially effective since ascidians accumulated again over time. However, due to the repetition of this treatment and also due to the reduction in free-swimming ascidian larvae in the autumn, the results were satisfying. Hillock and Costello [40] reported a >50% mortality of *Styela clava* (Pallas, 1774) when they were exposed to the sun at ambient temperatures (14–29 °C) after 24 h and a 100% mortality after 48 h, whereas Hopkins et al. [41] reported a 100% mortality of *Ciona* spp. after 6–24 h of air exposure. Furthermore, other findings indicate that air exposure and freshwater immersion promoted a mean ascidian mortality of just 27% at the end of a 30-day period [42]. It has also been reported that ascidian age can significantly affect their resistance to treatments [43]. The air exposure of mussel socks supported the low recruitment rate of the ascidians on the socks, especially during high larvae presence in the water column (July and August). Washing mussel socks followed by air exposure was the most efficient treatment, as minor mussel mortality occurred.

Concerning the immersion trials, they were conducted in October when the ascidian reproductive season had passed and the attached ascidians on mussel socks had proliferated. Immersing mussel socks in 50 ppt and 70 ppt salinity solutions was not satisfying in terms of reducing the number of ascidian colonies. Ascidians may be resistant to changes in salinity or have hyperosmotic regulation mechanisms [44]. It has also been reported that brine baths with lower than 70 ppt salinity may be effective against ascidians, as fewer juvenile mussels are affected [45,46]. Brine baths of 32 and 70 ppt may be powerful against sponges and ascidians while also having no adverse impacts on bivalves such as oysters or mussels. Strategies for fouling control on aquaculture gear include salt brine dips for 10 min followed by air exposure for two hours, and this treatment seems to be effective for removing attached ascidians. Carver et al. [25] pointed out that exposure to saturated brine for 8 min was 20% effective for eradicating ascidians. Submerging individuals in a hypersaline solution treatment (60 ppt salinity) for 20 s promoted a mortality of 73.3 $\pm$ 6.7% in ascidians after 15 days, and all mussels survived this treatment, but there was no effect against *S. plicata* (Lesueur, 1823) [42].

The immersion of mussel socks in the 50 ppt or 70 ppt salinity solution had little or no effect on ascidian removal. Immersion in a 50 ppt salinity solution and then exposure to air also seems to provide inadequate results. Contrary to the above, immersing the mussel socks in a 70 ppt brine solution and then exposing them to air for 24 was effective for the removal of ascidians since it resulted in an ascidian mortality of 93%. These were the best results of our trials since 8 days after the treatment few colonies survived on the mussel socks. The 70 ppt salinity solution appears to represent a crucial point for the effectiveness of the method. The salinity solution and air exposure affect the osmoregulation mechanisms of the ascidians, leading to their mortality, while at the same time the mussels are not affected.

4.2. Strategies to Mitigate Biofouling in Shellfish Aquaculture

When fouling is seasonal, biofouling prevention by synchronizing husbandry practices with fouling patterns may be feasible [47]. Common fouling species may also vary in the depth at which they settle [48]. Another parameter to keep in mind is that ascidians have a very short reproduction cycle accompanied with rapid growth. The ascidian larvae are continuously present in the water column during the reproduction period, and the mussel

shells provide an ideal substrate for settlement and growth. Therefore, any treatment concerning the removal of ascidians from the mussel socks during their reproduction period aims at maintaining an acceptable farming condition of the mussel socks and not the entire cleanliness of the farm's infrastructure. Thus, frequent washing (15 to 30 days) seems to be an effective method of keeping the mussel socks in good condition. However, if both washing and air exposure are feasible, the ascidian reduction may be more effective. Moreover, immersing mussel socks in a high-salinity solution followed by air exposure at the end of the ascidian breeding season would reduce biofouling, leading to improved mussel health and growth. In addition, management practices can be adapted prior to periods of high biofouling loads to match to the needs of each farm. The ability to apply these practices more precisely may improve their effectiveness and subsequently reduce the overall production costs [12,49–52].

In general, many techniques have been used in the past for efficient ascidian alleviation, such as chemical treatments, washing, disinfection, anti-metamorphic substances, air exposure, freshwater and heat exposure [51–56]. However, in some of the aforementioned studies, high mortality levels of the cultured organisms were observed, making them unsuitable for commercial use [54,57]. Currently, although progress has been made, there is no completely successful eradication method that is free of limitations. This is mainly due to economic terms, as profit gains are lower than the treatment costs. In comparison with previous studies, here, no mortality was observed in cultured organisms, increasing the efficiency of the studied method. Further, taking into consideration both the importance of *M. galloprovincialis* farming in the Mediterranean and the physiology of the species, there are some methods that cannot be applied in this type of farming. More specifically, the epibiont removal that has been applied in *Argopecten purpuratus* seems impossible for *M. galloprovincialis*, as the *M. galloprovincialis* broodstock originates mainly from natural populations [58], in contrast to many oyster species that come from commercial facilities. Apart from mechanical limitations, chemical treatments have been proven to cause increases in stress in the cultured organisms, resulting in hazardous effects for the cultured organisms [59]. Furthermore, heat exposure, although effective, has been found to cause shellfish mortality as well [25], and it proved to be suitable only for some ascidian species [56]. One additional pro of the present approach concerns the combination of more than one treatment that can act synergistically towards the effectiveness against more ascidian taxa [60]. From the above, it can be concluded that there is no black and white solution regarding biofouling treatment. Thus, as no universal method exists, the limitations of each one should be further re-examined. The benefit of this apparent "success" in eliminating most ascidians and other biofouling agents with respect to the potential presence, transport and introduction of non-indigenous/invasive species is that the types of methodologies proposed here might also mitigate the spread of such species elsewhere [61].

In the present study, a rather simple but at the same time significant method was proposed that led to successful ascidian handling while keeping the survival rate of the cultured mussels at 100%. Further, it should be highlighted that the present study was applied in field conditions, providing a clearer picture in comparison with studies conducted under laboratory settings. Overall, the proposed methods are mainly applicable in *Mytilus* mussels farming, in which removal of the mussels from the water is a routine action 1–2 times between the initial placement of mussels on the farm and harvest. These methods would likely require high costs in other types of farmed bivalves and would not be suggested in those cases. In reared mussels, this technique is cost efficient and thus practicable.

5. Conclusions

In summary, we proposed here a highly effective method for mitigating fouling organisms in mussel aquaculture. We observed that the combination of mussel sock immersion in a 70 ppt solution followed by air exposure for 24 h was the most efficient method and led to a 93% eradication of ascidians, combined with the avoidance of mortality

of the cultured organisms. As *M. galloprovincialis* represent the major cultivated bivalve species in the Mediterranean Sea, the development of an efficient method for their successful culture is of utmost importance. However, there are some limitations, such as labor demands and that the proposed method is species-oriented, taking into consideration the biological characteristics of *M. galloprovincialis*. As there is a lack of a completely efficient anti-fouling treatment method (especially considering labor, time and cost demands and the cultured organism's physiology), there is a need for a re-evaluation of the limitations that also considers the operational costs. Although labor-intensive and time-consuming, this method proved to be rather simple and beneficial both for the alleviation of ascidians and the survival of the culture organisms. Further, it needs to be highlighted that the proposed method can be immediately applicable for on-site applications.

Author Contributions: Conceptualization, D.T. and J.A.T.; methodology, D.T. and A.L.; software, D.K.P. and K.F.; validation, M.V.A. and I.G.; formal analysis, M.V.A. and I.A.G.; investigation, D.T. and B.M.; resources, J.A.T.; data curation, K.F. and I.A.G.; writing—original draft preparation, D.T. and D.K.P.; writing—review and editing, I.A.G., K.F. and J.A.T.; visualization, B.M. and J.A.T.; supervision, B.M. and J.A.T.; project administration, I.A.G. and J.A.T.; funding acquisition, B.M. and J.A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the EU-GREECE OPERATIONAL PROGRAM OF FISHERIES, EPAL 2014–2020, grant number MIS: 5048463. The present work is a part of the project 'Development of the best control practices of invasive ascidians in mussel farming infrastructures and remediation of economic effects of invasion'.

Institutional Review Board Statement: The study did not require ethical approval since it concerns animals that do not require ethical approval for their study according to Directive 2010/63/EU.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the reported results of this study are included in the manuscript.

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

References

1. Khosravi, M.; Nasrolahi, A.; Shokri, M.R.; Dobretsov, S.; Pansch, C. Impact of warming on biofouling communities in the northern Persian Gulf. *J. Therm. Biol.* **2019**, *85*, 102403. [CrossRef]
2. Lane, A.; Willemsen, P.R. Collaborative effort looks into biofouling. *Fish Farming Int.* **2004**, *44*, 34–35.
3. Enright, C. Control of fouling in bivalve aquaculture. *World Aquacult.* **1993**, *24*, 44–46.
4. Claereboudt, M.R.; Bureau, D.; Côté, J.; Himmelman, J.H. Fouling development and its effect on the growth of juvenile giant scallops (*Placopecten magellanicus*) in suspended culture. *Aquaculture* **1994**, *121*, 327–342. [CrossRef]
5. Aarnes, J.V.; Rudi, H.; Loland, G. Chap. 12: Current forces on cage, net deflection. In *Engineering for Offshore Fish Farming*; Thomas Telford Publishing: London, UK, 1990; pp. 137–152. [CrossRef]
6. Feidantsis, K.; Papadopoulos, D.K.; Lattos, A.; Theodorou, J.A.; Michaelidis, B.; Giantsis, I.A. Effects of Biofouling by Ascidians on Cultured Mussels: Apoptosis, Autophagy, and Antioxidant Defense. *J. Shellfish. Res.* **2008**, *42*, 199–213. [CrossRef]
7. Papadopoulos, D.K.; Lattos, A.; Giantsis, I.A.; Theodorou, J.A.; Michaelidis, B.; Feidantsis, K. The impact of ascidian biofouling on the farmed Mediterranean mussel *Mytilus galloprovincialis* physiology and welfare, revealed by stress biomarkers. *Biofouling* **2023**, *39*, 271–288. [CrossRef] [PubMed]
8. Ramsay, A.; Davidson, J.; Landry, T.; Arsenault, G. Process of invasiveness among exotic tunicates in Prince Edward Island, Canada. *Biol. Invasions* **2008**, *10*, 1311–1316. [CrossRef]
9. Daigle, R.M.; Herbinger, C.M. Ecological interactions between the vase tunicate (*Ciona intestinalis*) and the farmed blue mussel (*Mytilus edulis*) in Nova Scotia, Canada. *Aquat. Invasions* **2009**, *4*, 177–187. [CrossRef]
10. Adams, C.M.; Shumway, S.E.; Whitlatch, R.B.; Getchis, T. Biofouling in marine molluscan shellfish aquaculture: A survey assesses the business and economic implications of mitigation. *J. World Aquacult Soc.* **2011**, *42*, 242–252. [CrossRef]
11. Mckindsey, C.W.; Landry, T.; O'beirn, F.; Davies, I.M. Bivalve aquaculture and exotic species: A review of ecological considerations and management issues. *Shellfish Res.* **2007**, *26*, 281–294. [CrossRef]
12. Tsotsios, D.; Moutopoulos, D.K.; Lattos, A.; Michaelidis, B.; Theodorou, J.A. Impacts of the establishment of biofoulants on greek aquaculture: Farmers' Expert knowledge. *J. Mar. Sci. Eng.* **2023**, *11*, 1077. [CrossRef]
13. Fitridge, I.; Dempster, T.; Guenther, J.; de Nys, R. The impact and control of biofouling in marine aquaculture: A review. *Biofouling* **2012**, *28*, 649–669. [CrossRef]

14. Ramsay, A.; Davidson, J.; Bourque, D.; Stryhn, H. Recruitment patterns and population development of the invasive ascidian *Ciona intestinalis* in Prince Edward Island, Canada. *Aquat. Invasions* **2009**, *4*, 169–176. [CrossRef]
15. Turon, X. Periods of non-feeding in Polysyncratonlacazei (Ascidiacea: Didemnidae): A rejuvenative process? *Mar. Biol.* **1992**, *112*, 647–655. [CrossRef]
16. Perez-Portela, R.; Palacin, C.; Duran, S.; Turon, X. Biological traits of three closely related species of *Pycnoclavella* (*Ascidiacea*) in the Western Mediterranean. *Mar. Biol.* **2007**, *152*, 1031–1038. [CrossRef]
17. Bates, W.R. Environmental factors affecting reproduction and development in ascidians and other prochordates. *Can. J. Zool.* **2005**, *83*, 51–61. [CrossRef]
18. Lambert, G. Ecology and natural history of the protochordates. *Can. J. Zool.* **2005**, *83*, 34–50. [CrossRef]
19. Goodbody, I. Continuous breeding in three species of tropical ascidians. *Proc. Zool. Soc. Lond.* **1961**, *136*, 403–409. [CrossRef]
20. Stoner, D.S. Recruitment of a tropical colonial ascidian: Relative importance of pre-settlement vs. post-settlement processes. *Ecology* **1990**, *71*, 1682–1690. [CrossRef]
21. Lacoste, E.; Gaertner-Mazouni, N. Biofouling impact on production and ecosystem functioning: A review for bivalve aquaculture. *Rev. Aquac.* **2015**, *7*, 187–196. [CrossRef]
22. Bannister, J.; Sievers, M.; Bush, F.; Bloecher, N. Biofouling in marine aquaculture: A review of recent research and developments. *Biofouling* **2019**, *35*, 631–648. [CrossRef] [PubMed]
23. Cox, B.; Kosmeyer, P.; O'Conoe, W.; Dove, M.; Johnstone, K. Oyster Overcatch: Cold Shock Treatment. 2012. Available online: https://www.frdc.com.au/sites/default/files/products/2010-734-DLD.pdf (accessed on 10 March 2024).
24. Paetzold, S.C.; Davidson, J. Aquaculture fouling: Efficacy of potassium monopersulphonate triple saltbased disinfectant (Virkon® Aquatic) against *Ciona intestinalis*. *Biofouling* **2011**, *27*, 655–665. [CrossRef]
25. Carver, C.E.; Chisholm, A.; Mallet, A.L. Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. *J. Shellfish Res.* **2003**, *22*, 621–631.
26. Arens, C.; Paetzold, C.; Ramsay, A.; Davidson, J. Pressurized seawater as an antifouling treatment against the colonial tunicates *Botrylloides violaceus* and *Botryllus schlosseri* in mussel aquaculture. *Aquat. Invasions* **2011**, *6*, 465–476. [CrossRef]
27. Paetzold, S.C.; Hill, J.; Davidson, J. Efficacy of high-pressure seawater spray against colonial tunicate fouling in mussel aquaculture: Inter-annual variation. *Aquat. Invasions* **2012**, *7*, 555–566. [CrossRef]
28. Curtis, L.J.F.; Pearce, C.M.; Hodes, V.; Nelson, J.C.; Wasser, C.; Savery, J.; Therriault, T.W. Mitigating non-indigenous species movements: Effects of pressure-washing intensity and duration on the removal of biofouling and mobile invertebrates from cultured Pacific oysters (*Crassostrea gigas* (Thunberg, 1793)). *Manag. Biol. Invasions* **2021**, *12*, 618–639. [CrossRef]
29. Sievers, M.; Fitridge, I.; Bui, S.; Dempster, T. To treat or not to treat: A quantitative review of the effect of biofouling and control methods in shellfish aquaculture to evaluate the necessity of removal. *Biofouling* **2017**, *33*, 755–767. [CrossRef]
30. Cahill, L.P.; Atalah, J.; Cunningham, S.; Day, A.; Fletcher, L.; South, P.; Forrest, B.; Hopkins, G. Acetic acid immersion—A reactive pest treatment for bivalve aquaculture. *Aquaculture* **2021**, *533*, 736173. [CrossRef]
31. Katselis, G.N.; Moutopoulos, D.K.; Dimitriou, E.N.; Koutsikopoulos, C. Long-term changes of fisheries landings in enclosed gulf lagoons (Amvrakikos gulf, W Greece): Influences of fishing and other human impacts. *Est. Coast. Shelf Sci.* **2013**, *131*, 31–40. [CrossRef]
32. Kountoura, K.; Zacharias, I. Temporal and spatial distribution of hypoxic/seasonal anoxic zone in Amvrakikos Gulf, Western Greece. *Est. Coast. Shelf Sci.* **2011**, *94*, 123–128. [CrossRef]
33. Kountoura, K.; Zacharias, I. Trophic state and oceanographic conditions of Amvrakikos Gulf: Evaluation and monitoring. *Desalination Water Treat.* **2013**, *51*, 2934–2944. [CrossRef]
34. Nicolaidou, A.; Moraitou-Apostolopoulou, M.; Ignatiades, L. A survey of estuarine benthic, zooplanktonic and phytoplanktonic communities of Amvrakikos Gulf, Ionian Sea. *Mar. Ecol.—Pubbl. Stn. Zool. Napoli* **1983**, *4*, 197–209. [CrossRef]
35. Panayotidis, P.; Pancucci, M.A.; Balopoulos, E.; Gotsis-Skretas, O. Plankton distribution patterns in a Mediterranean dilution basin: Amvrakikos Gulf (Ionian Sea, Greece). *Mar. Ecol.—Pubbl. Stn. Zool. Napoli* **1994**, *15*, 93–104. [CrossRef]
36. Friligos, N.; Balopoulos, E.T.; Psillidou-Giouranovits, R. Eutrophication and hydrography in the Amvrakikos Gulf, Ionian Sea. *Fresenius Environ. Bull.* **1997**, *6*, 21–26.
37. Ferentinos, G.; Papatheodorou, G.; Geraga, M.; Iatrou, M.; Fakiris, E.; Christodoulou, D.; Dimitriou, E.; Koutsikopoulos, C. Fjord water circulation patterns and dysoxic/anoxic conditions in a Mediterranean semi-enclosed embayment in the Amvrakikos Gulf, Greece. *Est. Coast. Shelf Sci.* **2010**, *88*, 473–481. [CrossRef]
38. Kehagias, G.; Aposporis, M. Zooplankton variation in relation to hydrology in an enclosed hypoxic bay (Amvrakikos Gulf, Greece). *Mediterr. Mar. Sci.* **2014**, *15*, 554–568. [CrossRef]
39. Ordóñez, V.; Pascual, M.; Fernández-Tejedor, M.; Turon, X. When invasion biology meets taxonomy: *Clavelina oblonga* (Ascidiacea) is an old invader in the Mediterranean Sea. *Biol. Invasions* **2016**, *18*, 1203–1215. [CrossRef]
40. Hillock, K.A.; Costello, M.J. Tolerance of the invasive tunicate *Styela clava* to air exposure. *Biofouling* **2013**, *29*, 1181–1187. [CrossRef] [PubMed]
41. Hopkins, G.A.; Prince, M.; Cahill, P.L.; Fletcher, L.M.; Atalah, J. Desiccation as a mitigation tool to manage biofouling risks: Trials on temperate taxa to elucidate factors influencing mortality rates. *Biofouling* **2016**, *32*, 1–11. [CrossRef]

42. Santos, P.M.; Venâncio, E.; Dionísio, M.A.; Heumüller, J.; Chainho, P.; Pombo, A. Comparison of the Efficiency of Different Eradication Treatments to Minimize the Impacts Caused by the Invasive Tunicate *Styela plicata* in Mussel Aquaculture. *Animals* **2023**, *13*, 1541. [CrossRef]
43. Darbyson, E.; Locke, A.; Hanson, J.M.; Willison, J.M. Marine boating habits and the potential for spread of invasive species in the Gulf of St. Lawrence. *Aquat. Invasions* **2009**, *4*, 87–94. [CrossRef]
44. Sims, L.L. Osmoregulatory capabilities of three macrosympatrics to lidobranch ascidians, *Styela clava* Herdman, *S. plicata* (Lesueur), and *S. montereyensis* (Dall). *J. Exp. Mar. Biol. Ecol.* **1984**, *82*, 117–129. [CrossRef]
45. Carman, M.R.; Morris, J.A.; Karney, R.C.; Grunden, D.W. An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. *J. Appl. Ichthyol.* **2010**, *26*, 8–11. [CrossRef]
46. Carman, M.R.; Lindell, S.; Green-Beach, E.; Starczak, V.R. Treatments to eradicate invasive tunicate fouling from blue mussel seed and aquaculture socks. *Manag. Biol. Invasions* **2016**, *7*, 101–110. [CrossRef]
47. Sievers, M.; Dempster, T.; Fitridge, I.; Keough, M.J. Monitoring biofouling communities could reduce impacts to mussel aquaculture by allowing synchronization of husbandry techniques with peaks in settlement. *Biofouling* **2014**, *30*, 203–212. [CrossRef] [PubMed]
48. Woods, C.M.C.; Floerl, O.; Hayden, B.J. Biofouling on Greenshell (TM) mussel (*Perna canaliculus*) farms: A preliminary assessment and potential implications for sustainable aquaculture practices. *Aquac. Int.* **2012**, *20*, 537–557. [CrossRef]
49. Berntsson, K.M.; Jonsson, P.R. Temporal and spatial patterns in recruitment and succession of a temperate marine fouling assemblage: A comparison of static panels and boat hulls during the boating season. *Biofouling* **2003**, *19*, 187–195. [CrossRef] [PubMed]
50. Guenther, J.; Fitridge, I.; Misimi, E. Potential antifouling strategies for marine finfish aquaculture: The effects of physical and chemical treatments on the settlement and survival of the hydroid *Ectopleura larynx*. *Biofouling* **2011**, *27*, 1033–1042. [CrossRef] [PubMed]
51. Paetzold, S.C.; Davidson, J. Viability of golden star tunicate fragments after high-pressure water treatment. *Aquaculture* **2010**, *302*, 91–92. [CrossRef]
52. Cahill, P.; Heasman, K.; Jeffs, A.; Kuhajek, J.; Mountfort, D. Preventing ascidian fouling in aquaculture: Screening selected allelochemicals for anti-metamorphic properties in ascidian larvae. *Biofouling* **2012**, *28*, 39–49. [CrossRef]
53. Forrest, B.M.; Hopkins, G.A.; Dodgshun, T.J.; Gardner, J.P.A. Efficacy of acetic acid treatments in the management of marine biofouling. *Aquaculture* **2007**, *262*, 319–332. [CrossRef]
54. LeBlanc, N.; Davidson, J.; Tremblay, R.; McNiven, M.; Landry, T. The effect of antifouling treatments for the clubbed tunicate on the blue mussel, *Mytilus edulis*. *Aquaculture* **2007**, *264*, 205–213. [CrossRef]
55. Denny, C.M. Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. *ICES J. Mar. Sci.* **2008**, *65*, 805–810. [CrossRef]
56. Sievers, M.; Dempster, T.; Keough, M.J.; Fitridge, I. Methods to prevent and treat biofouling in shellfish aquaculture. *Aquaculture* **2019**, *505*, 263–270. [CrossRef]
57. de Nys, P.C.; Steinberg, P.D.; Charlton, T.S.; Christov, V. Antifouling of Shellfish and Aquaculture Apparatus. U.S. Patent US6692557B1, 17 February 2004.
58. Giantsis, I.A.; Mucci, N.; Randi, E.; Abatzopoulos, T.J.; Apostolidis, A.P. Microsatellite variation of mussels (*Mytilus galloprovincialis*) in central and eastern Mediterranean: Genetic panmixia in the Aegean and the Ionian Seas. *JMBA* **2014**, *94*, 797–809. [CrossRef]
59. Park, M.S.; Kim, Y.D.; Kim, B.M.; Kim, Y.J.; Kim, J.K.; Rhee, J.S. Effects of antifouling biocides on molecular and biochemical defense system in the gill of the pacific oyster *Crassostrea gigas*. *PLoS ONE* **2016**, *11*, e0168978. [CrossRef]
60. Shin, J.H.; Lee, S.Y.; Dougherty, R.H.; Rasco, B.; Kang, D.H. Combined effect of mild heat and acetic acid treatment for inactivating Escherichia coli O157: H7, Listeria monocytogenes and Salmonella typhimurium in an asparagus puree. *J. Appl. Microbiol.* **2006**, *101*, 1140–1151. [CrossRef]
61. Piló, D.; Pereira, F.; Carvalho, A.N.; Vasconcelos, P.; Cunha, A.M.; Gaspar, M.B. Are non-indigenous species hitchhiking offshore farmed mussels? A biogeographic and functional approach. *Mar. Pollut. Bull.* **2021**, *171*, 112776. [CrossRef]