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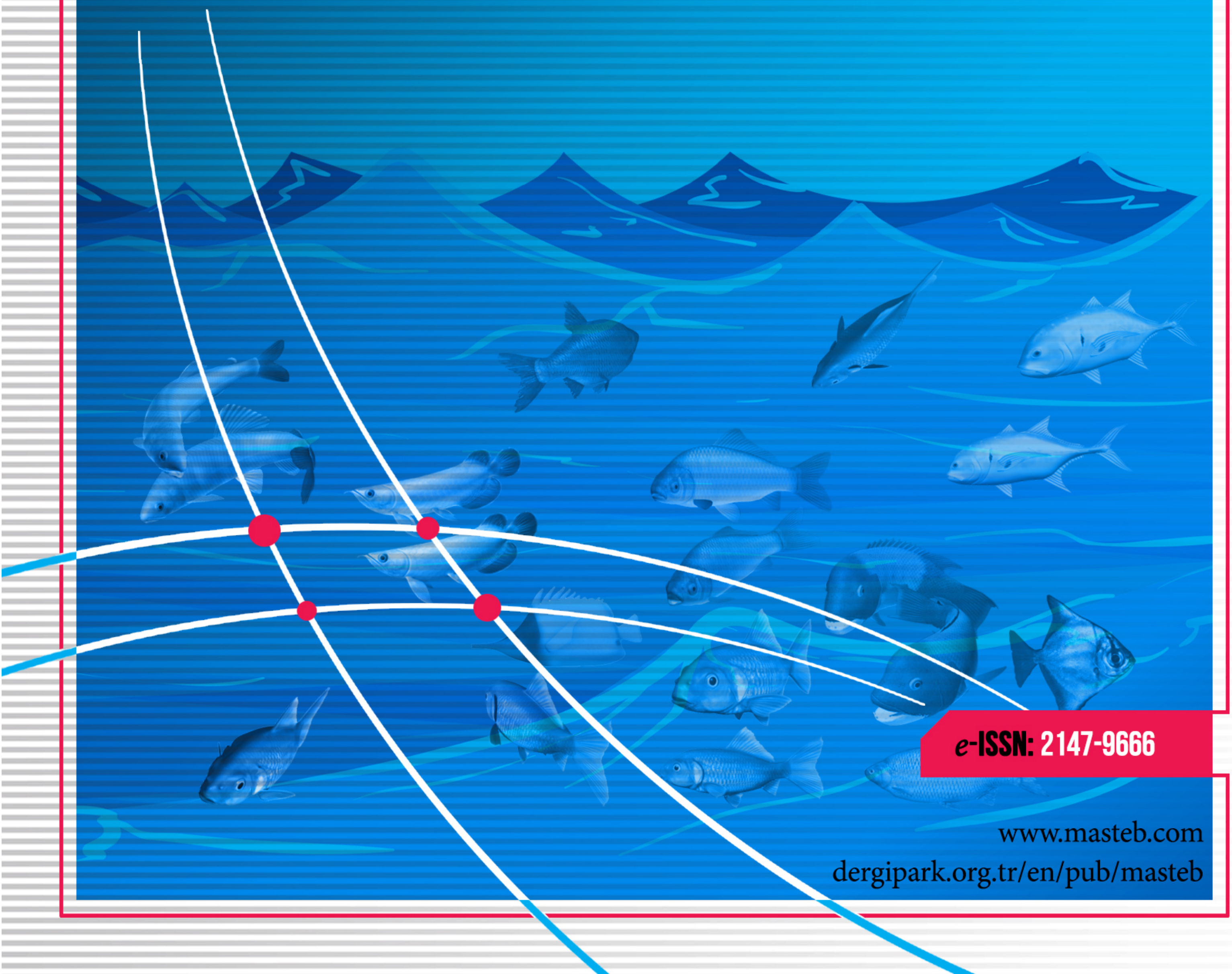

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RESEARCH ARTICLE

Investigation of the relationships between personality traits of leaders and accidents in the maritime sector with adaptation of the five-factor personality inventory into Turkish

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ABSTRACT

Despite the many developments in the maritime sector in recent years, the number of accidents is still at a significantly high level. Beyond the accident analysis, which is defined as the reactive process, it is clear that more studies are needed in this sector, taking into account the personal traits of the employees and with the foresight that these traits may cause accidents. Statistically significant relationships were determined in this analysis, as a result of the five-factor personality inventory adapted into Turkish and the questionnaire covering the accidents (Occupational accident and marine casualty) on the ships of 156 leaders (Masters, chief engineers and chief officers). Participants who don't have an 'occupational accident' history have higher conscientiousness and agreeableness personality traits than those who have occupational accident history; when considered as facets, it was understood that they had a higher sense of duty, self-discipline, and ideas. In addition, relationships were determined between 'marine casualty' and personality traits only in the context of facets. It is seen that participants who don't have marine casualty history have higher gregariousness and lower values compared to those who have marine casualty history. Evaluations were made and some conclusions were reached with the understanding that the relationships identified in this study are similar to the results of the research conducted in this field of different sectors.

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Introduction

Besides the fact that seafarers at ships have a different subculture from others (Maurizio, 2013) and are exposed to social isolation (Sampson & Thomas, 2003), the maritime sector which we can call a human system, shaped by the effects of some factors (technology, environment and organizational) on the performance of employees (Rothblum et al., 2002) is a sector which the human factor is largely (about 80%) effective in accidents (Bea et al., 1997).

There are many studies showings that there is a relationship between accidents that result from a combination of errors or chains of errors, including a human error by making the wrong decision, taking inappropriate action, or inaction (Rothblum et al., 2002) and personality traits that are relatively consistent and permanent, although each individual's unique mental, emotional, social and physical characteristics vary over time (Ewen, 2010).

In the literature review, it is seen that there are many studies that determine the relationship between personality traits and occupational accident (Thoms & Venkataraman, 2002; Cellar et al., 2001; Clarke & Robertson, 2005; Pourmazaherian et al., 2017), accident propensity (Koç et al., 2014), safety behaviors (Beus et al., 2015; Pourmazaherian et al., 2017) medical error and problem solving tendency (Babaei et al., 2018), job engagement (Öngöre, 2014), leadership (Özbağ, 2016), cultural intelligence and intercultural communication competence (Yeke & Semerciöz, 2016), etc.

This study is important because there are very few studies investigating the relationships between the personality traits of the leaders in the maritime sector and the accidents (e.g., Makarowski et al., 2020), and also it is the first study in this sector in Turkey.

Using the adaptation of the 'Five-Factor Personality Inventory', this study aims to examine whether there is a significant relationship or not between the personalities of leaders who are actively working at sea and occupational accidents or serious/very serious maritime accidents on their ships.

The 'International Personality Item Pool' which was developed by Goldberg (1999) was used to create the item pool of the questionnaire of this study (IPIP, 2019). The 'Five Factor Personality Inventory' questionnaire which was created by selecting the relevant items from the aforementioned pool with the support of linguists, professional experts and psychologists, was conducted with a group of 156 Turkish oceangoing masters, chief officers and chief engineers.

Materials and Methods

The survey method was adopted as the data collection method in this research. In the questionnaire created for the purposes of the research, there are 5 main factors and 3 facets for each main factor with 60 questions (reduced to 40 questions in the analysis phase). In this part of the study, validity and reliability findings for the mentioned inventory are included.

Demographic, Occupational and Descriptive Findings

The respondents are a very experienced sample group considering that 52.6% of them are over 40 years old, or in other words, 77.5% of them are over 35 years old. It can be seen that 53.8% of the participants are oceangoing masters, 25.6% of them are chief engineers and 20.5% of them are chief officers in terms of their ranks at the ship (Table 1).

Table 1. Demographic statistics

Item	Category	Frequency (n)	Percentage
Age	Between 24-29	4	2.6%
	Between 30-35	31	19.9%
	Between 36-39	39	25.0%
	Between 40-45	25	16.0%
	Between 46-50	21	13.5%
	Age 51 and over	36	23.1%
	Total	156	100.0%
Rank	Master	84	53.8%
	Chief Officer	32	20.5%
	Chief Engineer	40	25.6%
	Total	156	100.0%

When the occupational accidents and marine casualties that took place at ships of the participants are examined (Table 2), it is seen that 80.8% of them have an occupational accident history and 48.7% of them have a marine casualty history.

Personality Measurement Tool

First of all, some maritime companies were interviewed, with their written approval, the survey link was sent to the leaders working on their ships and these seafarers answered the questionnaire electronically of their own free will. All the information covering their answers has not been shared with anyone, including these mentioned companies. The respondents are still actively working at ships, and they are all Turkish citizens. The five-factor personality inventory which was adapted into Turkish was used only in this study, and the details about its validity and reliability are explained below.

Table 2. Occupational statistics

Item	Category	Frequency (n)	Percentage
How many "Occupational Accidents" occurred in your department during your leadership at ship (while you were Captain and/or Chief Officer or Chief Engineer)?	Never	30	19.2%
	1-2 times	80	51.3%
	3-5 times	32	20.5%
	6-10 times	8	5.1%
	11-15 times	4	2.6%
	More than 15	2	1.3%
	Total	156	100.0%
How many "Marine Casualty" occurred during your time leading at ship (while you were Captain and/or Chief Officer or Chief Engineer)?	Never	80	51.3%
	1-2 times	63	40.4%
	3-5 times	11	7.0%
	6-10 times	2	1.3%
	Total	156	100.0%

Table 3. Bartlett test of sphericity of each main factor, Cronbach's alpha value ranges of facets and ranges of items' factor scores

	Ranges of Items' Factor Scores	Bartlett Test of Sphericity	Cronbach's Alpha Value Ranges of Facets
Neuroticism	0.540-0.867	χ^2(15)=83.278 Sig.=0.000	0.534-0.636
Extraversion	0.479-0.861	χ^2(36)=179.517 Sig.=0.000	0.597-0.766
Agreeableness	0.431-0.874	χ2(45)=257.333 Sig.=0.000	0.563-0.707
Conscientiousness	0.512-0.888	χ2(28)=152.600 Sig.=0.000	0.582-0.709
Openness to experience	0.507-0.768	χ2(28)=84.175 Sig.=0.000	0.583-0.659

Explanatory Factor Analysis and *Cronbach's Alpha reliability* analyzes were applied separately for each main factor within the scope of validity and reliability analyzes of 5 different main factors belonging to the five-factor personality inventory. Before the explanatory factor analysis, *Kaiser Meyer Olkin* (KMO) *sampling adequacy value* and *Bartlett's Test of Sphericity* statistics were checked. Cronbach's Alpha reliability analyzes were applied to control reliability levels of the scale and facets.

The Bartlett test of sphericity values of each main factor, the Cronbach's alpha value ranges of the facets of each main factor and the factor score ranges of the items of the questionnaire are listed for this scale in Table 3.

As a result of the reliability analysis covering all the questionnaire questions, the Cronbach's alpha value of the developed scale was determined as 0.761. When Table 3 is examined, it can be seen that the Cronbach's alpha value ranges of the facets of main factors are between 0.534-0.766 and the factor score ranges of the items are between 0.431-0.888. As a result, it is understood that the validity and reliability levels of the scale which was developed are sufficient (Özdamar, 2016).

Findings Covering the Effects of Personality Traits on Accidents

The results of the independent sample t-test in terms of personality traits, which was applied to compare the five factors and their facets between ship leaders who have occupational accident history (126 people) and ship leaders who don't have an occupational accident (30 people) are provided in Table 4. When Table 4 is examined, agreeableness (t(154)=2.262, Sig.<0.05) and responsibility (t(154)=2.607, Sig.<0.05) as main factors; self-discipline (t(154)=2.353, Sig.<0.05), sense of duty (t(154)=2.658, Sig.<0.05) and ideas (t(154)=3.017, Sig.<0.05) as facets of factors draw attention. It can be seen for those traits that averages of ship leaders who don't have occupational accident history are higher than ship leaders who have occupational accident history; and there is a statistically significant difference between them at the 5% significance level.

The results of the independent sample t-test in terms of personality traits, which was applied to compare the five factors and their facets between ship leaders who have marine casualty history (76 people) and ship leaders who don't have marine casualty history (80 people) are given in Table 5.

Although no significant difference is found as the main factors between the personality traits of the leaders on board and marine casualty; gregariousness (t(154)=1.983, Sig.<0.05) and values (t(154)=-2.201, Sig.<0.05) as facets of factors (Table 5) are noticed. It can be seen for gregariousness that averages of ship leaders who don't have marine casualty history are higher than ship leaders who have marine casualty history; there is an opposite relationship for the facet of values, and there is a statistically significant difference between them at the 5% significance level.

Table 4. Independent sample t-test findings examining differences in main factors and their facets by occupational accident history

Trait	Occupational Accident History	N	$\overline{X}$	S.D.	t(154)	Sig.
Anger	Not have	30	4.383	0.838	1.927	0.056
	Have	126	4.028	0.924		
Anxiety	Not have	30	3.217	0.806	0.302	0.763
	Have	126	3.167	0.817		
Immoderation	Not have	30	3.867	0.694	0.988	0.324
	Have	126	3.714	0.773		
Neuroticism	Not have	30	3.822	0.529	1.666	0.098
	Have	126	3.636	0.554		
Gregariousness	Not have	30	3.544	0.652	1.260	0.209
	Have	126	3.331	0.872		
Assertiveness	Not have	30	4.544	0.406	1.900	0.059
	Have	126	4.349	0.526		
Excitement-Seeking	Not have	30	2.178	0.654	-0.856	0.393
	Have	126	2.315	0.816		
Extraversion	Not have	30	3.422	0.399	1.029	0.305
	Have	126	3.332	0.441		
Trust	Not have	30	4.050	0.708	1.921	0.057
	Have	126	3.776	0.701		
Modesty	Not have	30	3.133	0.805	0.917	0.360
	Have	126	2.979	0.835		
Altruism	Not have	30	4.450	0.578	1.689	0.093
	Have	126	4.242	0.612		
Agreeableness	Not have	**30**	**3.878**	**0.436**	**2.262***	**0.025**
	Have	**126**	**3.666**	**0.468**		
Self-Discipline	Not have	**30**	**4.517**	**0.676**	**2.353***	**0.020**
	Have	**126**	**4.143**	**0.805**		
Sense of Duty	Not have	**30**	**4.850**	**0.214**	**2.658***	**0.009**
	Have	**126**	**4.651**	**0.396**		
Competence	Not have	30	4.517	0.464	0.581	0.562
	Have	126	4.456	0.522		
Conscientiousness	Not have	**30**	**4.628**	**0.359**	**2.607***	**0.010**
	Have	**126**	**4.417**	**0.407**		
Ideas	Not have	**30**	**4.400**	**0.563**	**3.017***	**0.003**
	Have	**126**	**4.016**	**0.641**		
Emotionality	Not have	30	2.333	0.913	-1.326	0.187
	Have	126	2.552	0.784		
Values	Not have	30	3.356	0.955	0.886	0.377
	Have	126	3.190	0.909		
Openness to experience	Not have	30	3.363	0.427	1.184	0.238
	Have	126	3.253	0.466		

Note: * Indicates statistical significance at 5% significance level, $\overline{X}$: Mean, S.D.: Standard Deviation, t: T-test statistic, (Includes test degrees of freedom in parentheses), Sig.: Significance value.

Table 5. Independent sample t-test findings examining differences in main factors and their facets by marine casualty history

Trait	Marine Casualty History	N	$\bar{X}$	S.D.	t(154)	Sig.
Anger	Not have	80	4.206	0.920	1.547	0.124
	Have	76	3.980	0.903		
Anxiety	Not have	80	3.219	0.822	0.668	0.505
	Have	76	3.132	0.806		
Immoderation	Not have	80	3.838	0.783	1.594	0.113
	Have	76	3.645	0.725		
Neuroticism	Not have	80	3.754	0.518	1.921	0.057
	Have	76	3.586	0.578		
Gregariousness	Not have	**80**	**3.500**	**0.794**	**1.983***	**0.049**
	Have	**76**	**3.237**	**0.864**		
Assertiveness	Not have	80	4.383	0.517	-0.086	0.932
	Have	76	4.390	0.506		
Excitement-Seeking	Not have	80	2.271	0.796	-0.286	0.775
	Have	76	2.307	0.783		
Extraversion	Not have	80	3.385	0.476	1.056	0.293
	Have	76	3.311	0.384		
Trust	Not have	80	3.841	0.704	0.218	0.828
	Have	76	3.816	0.718		
Modesty	Not have	80	3.021	0.919	0.189	0.850
	Have	76	2.996	0.727		
Altruism	Not have	80	4.250	0.595	-0.673	0.502
	Have	76	4.316	0.626		
Agreeableness	Not have	80	3.704	0.459	-0.070	0.944
	Have	76	3.709	0.480		
Self-Discipline	Not have	80	4.213	0.787	-0.036	0.971
	Have	76	4.217	0.806		
Sense of Duty	Not have	80	4.722	0.360	1.118	0.265
	Have	76	4.655	0.392		
Competence	Not have	80	4.431	0.583	-0.921	0.358
	Have	76	4.507	0.420		
Conscientiousness	Not have	80	4.455	0.419	-0.065	0.948
	Have	76	4.459	0.394		
Ideas	Not have	80	4.158	0.639	1.371	0.172
	Have	76	4.018	0.644		
Emotionality	Not have	80	2.481	0.813	-0.446	0.656
	Have	76	2.539	0.816		
Values	Not have	**80**	**3.067**	**0.934**	**-2.201***	**0.029**
	Have	**76**	**3.386**	**0.874**		
Openness to experience	Not have	80	3.235	0.461	-1.073	0.285
	Have	76	3.314	0.457		

Note: * Indicates statistical significance at 5% significance level, $\bar{X}$: Mean, S.D.: Standard Deviation, t: T-test statistic, (Includes test degrees of freedom in parentheses), Sig.: Significance value.

Although a significant relationship could not be determined between age groups and occupational accidents, a significant relationship could be determined between age groups and maritime casualty. The Chi-Square test findings that test the differences in terms of marine casualty history according to age groups are presented in Table 6. When Table 6 is examined, it can be seen that the majority of the leaders in the age group over 46 have a history of marine casualty, while the majority of the leaders in the lower age groups do not have a history of marine casualty.

Serious marine casualties occur in very small numbers in the professional life of seafarers. It is understandable that these numbers increase with advancing age. For this reason, it is quite natural that ship leaders (especially aged 46 and over) who are definitely masters or chief engineers have more marine casualty backgrounds than chief officers.

Results and Discussion

It is understood that there is a negative relationship between the occupational accident histories of the leaders at ship and conscientiousness and agreeableness among the main factors. When considered in the context of facets of main factors, there is also a negative relationship between the occupational accident histories of the leaders and sense of duty, self-discipline, and ideas (Table 4).

No significant relationship could be found between the marine casualty and the five main personality traits of the leaders at the ship. However, some important relationships could be determined in the context of facets of some main factors. It can be seen that, while marine casualties are in a negative relationship with gregariousness, it is in a positive relationship with the values facet (Table 5).

Beus et al. (2015) examined the relationships between personality and unsafe behaviors, they found a negative relationship with conscientiousness and agreeableness, and a positive relationship with neuroticism and extraversion. According to this study, sensation seeking, altruism, anger and impulsiveness are also personality traits as facets that affect safety behaviors (Beus et al., 2015).

Cellar et al. (2001) investigated the relationships between workplace accidents and personality traits by self-reports of the participants, as in this study. They found an inverse relationship between only conscientiousness and agreeableness, and the accidents as main factors that are parallel to the relationships found in our study.

Clarke & Robertson (2005) discussed the relationship between personality traits and occupational/non-occupational accidents. According to this study, low conscientiousness and low agreeableness are effective personality effects in occupational accidents, while extroversion is only related to non-occupational accidents (such as traffic accidents).

Pourmazaherian et al. (2017) carried out a study in the construction sector, which is one of the risky sectors, and the authors indicated that neuroticism, conscientiousness and agreeableness are effective in both occupational and non-occupational accidents while conscientiousness and agreeableness personality traits are much more significant in improving safety performance.

Table 6. Chi-square test findings that test the differences in terms of marine casualty history by age groups

Age Group	Statistics	Marine Casualty History		Total
		Not Have	*Have*	
Between 24-35	Frequency (n)	25	10	35
	Percentage (%)	31.3%	13.2%	22.4%
Between 36-45	Frequency (n)	37	27	64
	Percentage (%)	46.3%	35.5%	41.0%
46+	Frequency (n)	18	39	57
	Percentage (%)	22.5%	51.3%	36.5%
Total	Frequency (n)	80	76	156
	Percentage (%)	100.0%	100.0%	100.0%
Chi-Square Test Statistics				
$\chi^2(02)=15.636^*$		Sig.=0.000		

Note: * Indicates statistical significance at 5% significance level, χ2: Chi-square test statistic, (Includes the degree of freedom of the chi-square test in parentheses.), Sig.: Significance value.

Many of the studies investigating the relationship between personality traits and accidents show that the conscientiousness factor is the most effective personality trait (Arthur & Graziano, 1996; Cellar et al., 2001; Clarke & Robertson, 2005). Christian et al. (2009) especially emphasized the conscientiousness personality trait as the determinant of safety performance in their meta-analytical path model.

A person who is deficient in planning and systematic decision making in unexpected situations has low conscientiousness and may be exposed to accidents. In addition, the reason for the low sense of duty under the conscientiousness personality trait may be related to the fact that a person is disrespectful to authority, socially incompatible and experiencing social deviation (West et al., 1993).

Like the conscientiousness personality trait, it is understood that people with low agreeableness also have more accidents history (Cellar et al., 2001; Clarke & Robertson, 2005). People with low agreeableness may be weaker in cooperating with others and may react more aggressively to situations. However, if individuals had high levels of agreeableness; they would both comply more with safety-related corporate policies and care more about the safety of their colleagues (Drew, 2014).

Conclusion

Taking into account the many risks in the maritime industry, it is understandable that people working at ships have much higher rates of injury and death compared to those working on the land (Borch et al., 2012; Roberts et al., 2014). Beyond the mere reactive process of incident investigations, this sector needs behavior-based approaches that are proactive and examine the situation before accidents occur (Rothblum et al., 2002). There is also a need for human-based safety approaches based on understanding and even accepting internal feelings, needs and perceptions among employees (Geller, 2006).

Much more research is needed on the impact of personality traits for accidents in the maritime industry. If the selection of personnel in the leadership position for maritime companies becomes widespread by conducting personality tests and maritime schools choose students based on their personality traits, it may allow a reduction in the number of accidents or deaths in the long run.

In addition, it may be useful to conduct an international survey with more ship leaders to determine the personality-accident relationship, including near misses which are the precursors of an accident.

As a final recommendation, it should be ensured that the system used to detect non-technical skills in aviation is also applied in maritime sector especially during promotions.

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Compliance With Ethical Standards

Authors' Contributions

HSÇ: Validation, investigation, data curation, writing original draft, methodology, visualization and conceptualization.

LT: Writing-review-editing and supervision.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The study has been approved by Istanbul Technical University Social and Human Sciences Human Research Ethics Committee on 25 February 2021. Project number: 156.

References

Arthur, W. Jr., & Graziano, W. (1996). The five-factor model, conscientiousness, and driving accident involvement. *Journal of Personality*, *64*(3), 593-618. https://doi.org/10.1111/j.1467-6494.1996.tb00523.x

Babaei, M., Mohammadian, M., Abdollahi, M., & Hatami, A. (2018). Relationship between big five personality factors, problem solving and medical errors. Heliyon, 4*(9)*, e00789. https://doi.org/10.1016/j.heliyon.2018.e00789

Bea, R. G., Holdsworth, R. D., & Smith, C. (1997). Human and organizational factors in the safety of offshore platforms. In R. G. Bea, R. D. Holdsworth, & C. Smith (Eds.), *1996 International Workshop on Human Factors in Offshore Operations* (pp. 71-84). American Bureau of Shipping.

Beus, J. M., Dhanani, L. Y., & McCord, M. A. (2015). A meta-analysis of personality and workplace safety: Addressing unanswered questions. *Journal of Applied Psychology, 100*(2), 481–498. https://doi.org/10.1037/a0037916

Borch, D. F., Hansen, H. L., Burr, H., & Jepsen, J. R. (2012). Surveillance of maritime deaths on board Danish merchant ships, 1986-2009. *International Maritime Health, 63*(1), 7-16.

Cellar, D. F., Nelson, Z. C., Yorke, C. M., & Bauer, C. (2001). The five-factor model and safety in the workplace: Investigating the relationships between personality and accident involvement. *Journal of Prevention & Intervention in the Community, 22*(1), 43-52. https://doi.org/10.1080/10852350109511210

Christian, M., Bradley, J., Wallace, J., & Burke, M. (2009). Workplace safety: A meta-analysis of the roles of person and situation factors. *Journal of Applied Psychology, 94*(5), 1103-1127. https://doi.org/10.1037/a0016172

Clarke, S., & Robertson, I. (2005). A meta-analytic review of the Big Five personality factors and accident involvement in occupational and non-occupational settings. *Journal of Occupational and Organizational Psychology, 78*(3), 355-376. https://doi.org/10.1348/096317905X26183

Drew, E. N. (2014). Personnel selection, safety performance, and job performance: Are safe workers better workers? [Ph.D. Thesis. Florida International University].

Ewen, R. B. (2010). *An Introduction to Theories of Personality* (7th ed.). Psychology Press.

Geller, E. S. (2006). People-based safety, an evolution of behavior-based safety for greater effectiveness. *Proceedings of the ASSE Professional Development Conference and Exposition*. Washington, pp. 12-13.

Goldberg, L. R. (1999). A broad-bandwidth, public-domain, personality inventory measuring the lower-level facets of several five-factor models. In Mervielde, I., Deary, I., De Fruyt, F., & Ostendorf, F. (Eds.), *Personality Psychology in Europe* (pp. 7-28). Tilburg University Press.

IPIP. (2019, September 23). *International Personality Item Pool.* Retrieved on January 15, 2020, from https://ipip.ori.org

Koç, M., Ayancı, M., Çolak, T. S., & Düşünceli, B. (2014). Kaza sayılarına göre kişilik profili: Kazaya yatkınlık. *5. Karayolu Trafik Güvenliği Sempozyumu ve Sergisi.* Turkey. pp. 318-328.

Makarowski, R., Plopa, M., Piotrowski, A., & Plopa, W. (2020). The human factor in maritime transport: personality and aggression levels of master mariners and navigation students. *Advances in Cognitive Psychology, 16*(4), 363-369. https://doi.org/10.5709%2Facp-0310-5

Maurizio, E. (2013). The social world of seafarers, A sociological research in central Italy. *Advances in Applied Sociology, 3*(4), 199-205. https://doi.org/10.4236/aasoci.2013.34027

Öngöre, Ö. (2014). A study of relationship between personality traits and job engagement. *Procedia - Social and Behavioral Sciences, 141*, 1315–1319. https://doi.org/10.1016/j.sbspro.2014.05.226

Özbağ, G. K. (2016). The role of personality in leadership: Five factor personality traits and ethical leadership. *Procedia - Social and Behavioral Sciences, 235*, 235-242. https://doi.org/10.1016/j.sbspro.2016.11.019

Özdamar, K. (2016). *Ölçek ve Test Geliştirme Yapısal Eşitlik Modeli.* Nisan Yayın Evi.

Pourmazaherian, M., Mohammed, S. Baqutayan, S., & Idrus, D. (2017). The role of the big five personality factors on accident: A case of accidents in construction industries. *Journal of Science, Technology and Innovation Policy, 7*(1), 34-43. https://doi.org/10.11113/jostip.v7n1.65

Roberts, S. E., Nielsen, D., Kotłowski, A., & Jaremin, B. (2014). Fatal accidents and injuries among Merchant seafarers worldwide. *Occupational Medicine, 64*(4), 259–266. https://doi.org/10.1093/occmed/kqu017

Rothblum, A., Wheal, D., Withington, S., Shappell, S. A., Wiegmann, D. A., Boehm, W., & Chaderjian, M. (2002). Improving incident investigation through inclusion of human factors. *2nd International Workshop on Human Factors in Offshore Operations.* United States Department of Transportation - Publications & Papers. 32.

Sampson, H., & Thomas, M. (2003). The social isolation of seafarers: causes, effects, and remedies. *International Maritime Health, 54*(1-4), 58-67.

Thoms, P., & Venkataraman, R. (2002). Relation of Managers Personality to Accident and Injury Rates. *Psychological Reports, 91*(3 Pt 2), 1107-1115.

West, R., Elander, J., & French, D. (1993). Mild social deviance, Type-A behaviour pattern and decision-making style as predictors of self-reported driving style and traffic accident risk. *British Journal of Psychology, 84*(Pt 2), 207-219. https://doi.org/10.1111/j.2044-8295.1993.tb02474.x

Yeke, S., & Semerciöz, F. (2016). Relationships between personality traits, cultural intelligence and intercultural communication competence. *Procedia - Social and Behavioral Sciences, 235*, 313-319. https://doi.org/10.1016/j.sbspro.2016.11.036

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REVIEW ARTICLE

Seaweeds: Bioactive components and properties, potential risk factors, uses, extraction and purification methods

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ABSTRACT

Seaweeds, also known as macroalgae, are abundant sources of various vital bioactive components with a wide range of biological functions. They are sold commercially and are primarily used in the food industry, pharmaceuticals, cosmeceuticals, and other related industries. The diverse biological activities linked with bioactive compounds obtained from seaweeds have the potential to expand their health benefit value in the food and pharmaceutical industries. Studies revealed that seaweeds have the potential to be used as complementary medicine due to its variety of biological properties that have been shown to be therapeutic for health and disease management, such as antibacterial, anticoagulant, anticancer, antidiabetic, antiestrogenic, antihypertensive, antihyperlipidemic, antifungal, anti-inflammatory, antioxidant, antiobesity, antiviral, immunomodulatory, neuroprotective, thyroid stimulant, tissue healing properties, and many more. Although seaweeds are generally beneficial to humans, they may still pose possible health risks due to high iodine concentration and exposure to heavy metals and arsenic concentrations. However, information on this topic is still limited. With the great importance of seaweeds, various green extraction methods such as Microwave-assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), Pressurized Solvents Extraction (PSE) and Enzyme-ssisted Extraction (EAE) were used as an alternative to the conventional method to isolate bioactive components and further purified using chromatographic technique analysis to ensure the purity of the extract. This review covers the following topics: general structure and characteristics of seaweeds, seaweed production, bioactive components and properties of seaweed, possible risk factors of seaweeds, applications of seaweeds, extraction, and purification of seaweed extracts.

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Introduction

Since ancient times, natural products have played a significant role in diagnosing, treating, and preventing numerous ailments. The therapeutic characteristics of chemical compounds in natural products are optimized and augmented for human medical applications (Gnanavel et al., 2019). The plant-based and herbal medications generated from natural resources that are considered pure, healthy, and safe have grown in popularity over the years (Van Wyk & Prinsloo, 2020). As a result, several herbal-based pharmaceutical sources are now commercially accessible and offered as an alternative therapy and dietary supplement to treat various illnesses (Woo et al., 2012). In addition, the availability of novel metabolites with diverse uses such as cosmeceuticals, nutraceuticals, agrochemicals, medicals, and other relevant chemical industries has stimulated marine drug research in recent years (Rengasamy et al., 2020). It has been considered that the marine ecosystem is an excellent source of natural compounds with several functions (Hentati et al., 2020). Seaweeds are marine plant organisms capable of producing a wide range of active metabolites with a wide range of medical applications, which they also use to defend themselves against other invading species (Kolanjinathan et al., 2014). As results of these novel metabolites, seaweed has become one of the most important sources of natural components used in pharmaceuticals, accounting for 30% of the global market in 2018. It was expected to be greater than USD 10,486.8 billion (Rengasamy et al., 2020).

Seaweeds, or known as macroalgae, are marine photosynthetic, non-flowering plant-like organisms that are categorized into three major groups depending on their predominant pigment compositions, which are green (Chlorophyta), brown (Ochrophyta), and red (Rhodophyta) seaweeds (Baweja et al., 2016). They can be found all across the world's coastlines, from the warm tropics to the freezing and icy polar regions (Mahadevan, 2015). Seaweeds are commercially sold, with approximately 83% of its total global production is for direct human consumption (Mahadevan, 2015). They are commonly consumed in Asian countries as fresh, dried, or as ingredients in prepared foods (Kılınç et al., 2013). The remaining percentage is used as a source of phycocolloids extracted for the application in food (Fleurence., 2016), cosmetic (Morais et al., 2021), medical (Shelar et al., 2012) and other related industries (Hentati et al., 2020). There are 221 species of seaweeds are utilized in total, with 145 species used for food and 101 species used for phycocolloid synthesis (Fleurence et al., 2018). Seaweeds are also employed in aquaculture as probiotics (Vatsos & Rebours, 2015), animal feed additive (Makkar et al., 2016), fertilizer (Ruban & Govindasamy, 2018), and as water purifier (Arumugam et al., 2018). In this context, the goal of this article is to provide general information about seaweeds, including their biological features, potential therapeutic properties, potential risk factors, and some of the extraction methods used. Furthermore, due to their distinct metabolite contents, this study also investigates the relevance and applications of seaweeds as major marine bioresources in numerous industries.

Materials and Methods

This study reviewed the available articles regarding the information about seaweeds and their novel metabolites. The study searched the keywords: seaweeds, bioactive compounds, bioactive properties, extraction, and characterization methods in Google Scholar, Pubmed, Web of Science, Science Direct, Mendeley and Scopus databases from 2011 to 2021. Articles published after the date of this review were not considered. In addition, online thesis and conference proceedings were also taken into account.

General Structure and Characteristics of Seaweeds

Seaweeds are large and diverse group of macroscopic multicellular, benthic (some species), non-flowering, plant-like organisms found in the world's aquatic environments (Zamani et al., 2013; Kalasariya et al., 2021). They can be extremely small or very huge, reaching lengths of up to 60 meters (Yu et al., 2014). Although many seaweeds have plant-like features, they are still not classified as true plants because they lack a specialized vascular system (Yu et al., 2014; Kalasariya et al., 2021; Morais et al., 2021). This vascular system is an internal conducting system that connects all organs and distributes fluids, nutrients, and numerous signaling molecules throughout the plant body (Lucas et al., 2013). Seaweeds, on the other hand, receive nutrients straight from the seawater and hence do not require an internal conducting system (De San, 2012). Seaweeds are eukaryotic organisms (Charrier et al., 2017) , they are macroalgae and thalloids in nature, which means they have basic thallus structures but no real leaves, stems, or roots, unlike other terrestrial plants. They do, however, have roots-like structures known as holdfasts or rhizoids (Rao et al., 2019). Seaweeds, like terrestrial plants, serve vital roles in marine ecology as primary producers. They are photosynthetic organisms, which means they can transform sunlight energy into materials for growth (Sudhakar et al., 2018). Seaweeds include pigments such as chlorophyll, which aids in the absorption of sunlight for photosynthesis and is responsible for their green color (Chen et al., 2017). Other pigments found in seaweeds are responsible for their intriguing colors, such as red and brown seaweeds (Morais et al., 2021).

Basic Parts of Seaweed

Some seaweeds employ *holdfast*, a root-like structure used only for anchoring, to attach themselves to the ocean floor. Other seaweeds, such as floating seaweeds, may have floating or *air sacs* that maintain them above the water's surface and expose them to sunlight for photosynthesis. Another seaweed component is *stipe*, a stem-like structure that holds the blades to the water's surface. *Blades* are primary photosynthetic leaf-like flattened parts that absorb sunlight. Furthermore, *thallus* refers to the fundamental or advanced body-like structure of seaweeds that can conduct photosynthesis with all its parts (Figure 1) (Suryanarayana Murty & Banerjee, 2011; Baweja et al., 2016; Sudhakar et al., 2018).

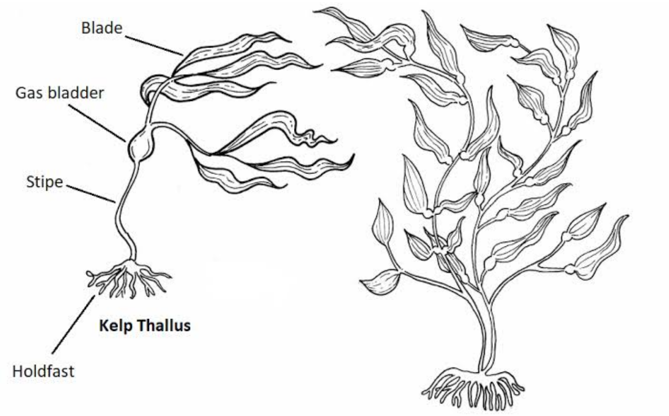

Figure 1. Basic parts of seaweed (Ha et al., 2021)

Classification of seaweeds

Based on their photosynthetic pigments, seaweeds are classified into three categories: brown (Ochrophyta or Class Phaeophyta), green (Chlorophyta), and red (Rhodophyta). There are around 10,000 seaweed species, including about 2000 brown, 1500 green, and 6500 red seaweeds (Collins et al., 2016; Gutiérrez-Rodríguez et al., 2018). Furthermore, seaweeds are classified using molecular techniques based on evolutionary processes (Figure 2) (Ruggiero et al., 2015).

Distribution of Seaweeds

With an average depth of 5 km, the water surface covers more than 70% of the earth's surface (Baweja et al., 2016), to which seawater accounts for more than 90% of all water on the planet earth (Sudhakar et al., 2018). Moreover, oceans support floating forests with a broad range of marine animals and plants, where the marine vegetation is more primordial and diversified than the terrestrial vegetation (Baweja et al., 2016). Algae are photosynthetic plant-like creatures that are the sole primary producers in the oceans (Sudhakar et al., 2018), with a photosynthetic efficiency (PE) that is 6–8% greater than that of terrestrial plants (1.8–2.2%) (Ashraf et al., 2016; Gutiérrez-Rodrguez et al., 2018). Seaweeds, also known as marine macroalgae or edible macroalgae, are benthic marine algae that thrive in brackish or saltwater environments (George & Mathew, 2017) found in shallow and open water up to 180m deep (Khalid et al., 2018). They are the most numerous marine vegetation and one of the essential biomass producers in the ocean that gives food and shelter to aquatic life (Ghadiryanfar et al., 2018). However, the distribution of seaweeds in the marine environment is limited only to the littoral and sublittoral zones. (Premarathna et al., 2019a). They may be found to a depth where 0.01% photosynthetic light is accessible (George & Mathew, 2017).

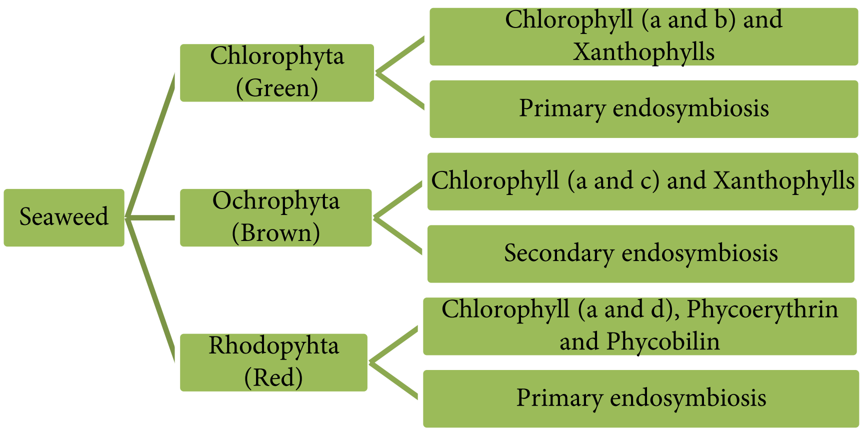

Figure 2. Classification of seaweeds

Table 1. Factors that affect the distribution of Seaweeds (adapted from Baweja et al., 2016)

Physical Parameters	Chemical Parameters	Biological Parameters
Substrate, temperature, light quality and quantity, dynamic tidal activity, winds, and storms.	Salinity, pH, nutrients, gases, and pollution level.	Herbivores, microbes, epiphytes, endophytes, symbionts, parasites, and diseases.

Table 2. Some pigment content of seaweeds (adapted from Yu et al., 2014)

Seaweeds	Pigments
Green seaweeds	α-, β-, and γ-carotene, chlorophylls a and b, lutein, siphonoxanthin, and siphonein
Brown seaweeds	Chlorophylls a, c1, and c2, β-carotene, and fucoxanthin
Red seaweeds	Chlorophyll a, r-phycocyanin, allophycocyanin, c-phycoerythrin, α- and β-carotene

Table 3. Chemical composition of seaweeds (% dry weight)

Species	Chemical composition of seaweed			
	Protein[a]	***Lipids***[b]	***Ash***[d]	***References***
Green seaweeds: *Caulerpa sertularioides, C. lentillifera, C. patentiramea, D. tenuissima, Ulva* sp., *Caulerpa* sp., *C. glomerata, Codium* sp., *Enteromorpha* sp., *Halimeda* sp., *M. oxyspermum, U. clathrata, U. lactuca*	32.7-3.3	13.04-1.57	27.5-19.59	Černá, 2011[a]; Mišurcová et al., 2011[b]; Gosch et al., 2012[b]; Fleurence et al., 2018[a]; Susanto et al., 2019[b]; Peñalver et al., 2020[d]
Brown seaweeds: *Costaria costata, D. bartayresii, D. dichotoma, H. fuziformis, Laminaria* sp., *S. japonica, Sargassum* sp., *S. macrodontum, A. nodusum, Dictyota* sp., *D. antartica, U. pinnatifida, Padina* sp., *Sargassum* sp., *B. bifurcata, F. vesiculosus, S. latissima, S. fusiforme, U.pinnatifida*	25.70-5.4	11.91-0.38	39.3-20.71	Černá, 2011[a]; Gosch et al., 2012[b]; Fleurence et al., 2018[a]; Susanto et al., 2019[b]; Peñalver et al., 2020[d]
Red seaweeds: *Laurencia papillosa, C. crassicaulis, C. yendoi, G. longissima, M. japonica, Palmaria* sp., *A. concinna, A. multifida, A. taxiformis, Bryothamnion* sp., *C. officinali, D. simplex, E. duperreyi, Eucheuma* sp., *G. acerosa, Gracilaria* sp., *G. turuturu, H. formousa, Hypnea* sp., *Chondrus* sp., *Laurencia* sp., *O. secundiramea, P. palmata, P. brasiliense, Porphyra* sp., *S. filiformi, V. obtusiloba, P. capillacea, J. rubens*	47.0-2.3	5.0-0.64	44.03-17.50	Černá, 2011[a]; Mišurcová et al., 2011[b]; Fleurence et al., 2018[a]; Susanto et al., 2019[b]; Peñalver et al., 2020[d]

In addition, their vertical and horizontal distribution differences demonstrate their adaptation to their surroundings. As a result, several species are confined to sheltered coves and bays, while others may be restricted to exposed cliffs along the coast or at the reef's margins. Different species of seaweeds may exist in a variety of transitional habitats; hence, the combined effects of various physical, chemical, and biological parameters on the distribution of seaweeds may determine the existence or absence of a species in a habitat (Table 1) (Baleta & Nalleb, 2016).

Production of Seaweeds

The Food and Agriculture Organization of the United Nations' recently published World State of Fisheries and Aquaculture Reports held biennially until 2018 (FAO, 2020). In the output of FAO, the total world fisheries and aquaculture production, roughly 54.1%, is represented by aquaculture, and in terms of overall world aquaculture production, coastal and marine aquaculture production accounted for around 55.2%. In the total coastal and marine aquaculture production, approximately 51.3% of it is represented by seaweeds with 32.4 million tons of total production, followed by mollusks with 17.3 million tons, approx. 27.4%, finfish with 7.3 million tons, approx. 11.6%, crustaceans with 5.7 million tons, approx. 9.1%, and other aquatic resources with 0.4 million tons, approx. 0.6% (Chopin & Tacon, 2021). In June 2021, FAO published a factsheet for the Global seaweeds and microalgae production from 1950-2019. According to the report, the global algal production, including cultivation and wild harvest, has increased more than 60 times since 1950, from 0.56 million tons of wet to 35.82 million tons in 2019. The increased in seaweed production is largely due to cultivation, which accounts for 34.74 million tons, or nearly 97% of total production, whereas natural harvesting produced only 1.08 million tons of wet (FAO, 2021a).

In 2019, around 97% of total world seaweed production (cultivated and wild) is centered in Asia, where China (20,351,442 tons, approx. 56.82%) is the top producer, followed by Indonesia (9,962,900 tons, approx. 27.81%), the Republic of Korea (1,821,475 tons, approx. 5.09%), the Philippines (1,500,326 tons, approx. 4.19%), the Democratic People's Republic of Korea (603,000 tons, approx. 1.68%), Japan (412,300 tons, approx. 1.15%), Malaysia (188,100 tons, approx. 0.53%). Other countries such as in Americas, Europe, Africa, and Oceania contributes only 1.36%, 0.08%, 0.41%, and 0.05% of the total world seaweeds production (cultivated and wild), respectively (FAO, 2021a). Species group of seaweeds such as brown seaweeds: *Laminaria/Saccharina* sp. (12,411,987 tons, approx. 34.65%), *Undaria* sp. (2,566,316 tons, approx. 7.16%), red seaweeds: *Kappaphycus/Eucheuma* sp. (11,685,174 tons, approx. 32.62%), *Gracilaria* sp. (3,695,231 tons, approx. 10.32%), and *Porphyra* sp. (2,984,573 tons, approx. 8.33%) are the top 5 species of seaweeds that contribute to the 99.84% of the world production of seaweeds both in wild and cultivation (FAO, 2021a). Furthermore, the value of the seaweed farming business might be far higher, particularly if monetary value is assigned to the ecological services supplied by seaweeds (Chopin & Tacon, 2021).

Bioactive components and biological properties of seaweeds

Seaweeds are renowned for their ability to produce a diverse range of biologically active macromolecules. Significant components of seaweeds are pigments, phenolic compounds, lipids, proteins, vitamins, minerals, and carbohydrates (polysaccharides) (Bedoux et al., 2014). Many studies have revealed that algae are the most abundant source of these bioactive chemicals, particularly polysaccharides that can be sulfated and non-sulfated (Jiménez-Escrig et al., 2011; Jesumani et al., 2019; Venugopal, 2019; Hentati et al., 2020; Rengasamy et al., 2020). Sulfated polysaccharides are represented by agars, carrageenans, fucoidans, and galactans, while non-sulfated polysaccharides are represented by alginates and laminaran (Rupérez et al., 2013; Hentati et al., 2020).

Table 4. Vitamin content (mg/100g dw) of seaweed (adapted from Škrovánková (2011) and Martínez–Hernández et al. (2018)).

Seaweeds	**Vitamin content**						
	E	***C***	***B12***	***B3***	***B2***	***B1***	***A***
Green seaweeds: *Caulerpa lentillifera, C. fragile, U. lactuca, U. pertusa, U.rigida, E. flexousa*	19.70-2	746.4-<0.223	142[a]	1.09-<0.5	0.559-0.02	4-< 0.02	9581-0.01
Brown seaweeds: *Alaria esculenta, F. vesiculosus, H. elongata, L. digitata, L. ochroleuca, S. japonica, S. latissima, U. pinnatifida, S. hemiphylum,*	3.43-1.4	785.1-153.8	99.1-4.4[a]	61.2-1.58	11.7-0.02	5-0.02	0.481-0.04
Red seaweeds: *Chandrus crispus, Gracilaria sp., G. changii, P. palmata, P. umbilicalis, P. yezoensis, K. alvarezii, Porphyra* sp.	13.9-0.267	711.9-107.1	760-122.4[a]	11.0-1.89	1.91-0.36	1.56-0.073	4.8-1.59

Note: [a] μg/kg

Pigments

Seaweeds include three forms of pigments: chlorophyll, carotenoid, and phycobiliproteins, all of which have great potential as ingredients for nutraceutical, as physiologically active agents due to their antiangiogenic, anticancer, antidiabetic, anti-inflammatory, antioxidant, and immunomodulatory characteristics, as well as used as food dyes. Chlorophylls are greenish pigments that are soluble in lipids and are essential for photosynthesis in seaweeds. The most common algal carotenoids include astaxanthin, carotenes, fucoxanthin, lutein, lycopene, neoxanthin, violaxanthin, and zeaxanthin. Carotenoids are tetrapenoid molecules that aid in seaweed photosynthesis. However, among these carotenoids, fucoxanthin, derived from brown seaweed, is the most prevalent, having potential applications in the food business. Finally, phycobiliproteins are pigments soluble in water and occur as proteins. Phycobiliproteins are composed of three different pigments: phycoerythrin is the most prevalent red pigment, allophycocyanin is the light-blue pigment, and phycocyanin is the blue pigment. These three pigments show different forms of protein, different bilin contents, and spectral properties (Table 2) (Aryee et al., 2018; Cherry et al., 2019).

Phenolic Compounds

Seaweed contains catechins, flavonoids, phenolic acids, phlorotannins, tannins, and other phenolic chemicals. Thus, seaweed species have a considerable effect on the kind and quantity of phenolic chemical extraction. Bromophenols, flavonoids, and phenolic acids are abundant in green and red seaweeds. Brown seaweeds have complex polymers mostly of phlorotannins and phloroglucinol oligomers (1,3,5-trihydroxy benzene). Seaweed polyphenols have been linked to many biological activities, including antibacterial, anticancer, antidiabetic, anti-inflammatory, antiobesity, antioxidant, antiproliferative, antitumor, and antiviral effects (Montero et al., 2017; Gómez-Guzmán et al., 2018; Cotas et al., 2021).

Lipids

The majority of seaweeds have low lipid concentrations of up to 5% by weight of the dry weight (DW) sample (Table 3) (Mišurcová et al., 2011). However, there are a number of species with total lipid content greater than 10% dw (Table 3), making them viable candidates for oil-based goods (Gosch et al., 2012). The total lipid content varies according to geographical location, interactions, light intensity, salinity, seasonal change, species, and temperature (Susanto et al., 2019). Seaweed lipids, on the other hand, include large quantities of Polyunsaturated Fatty Acids (PUFAs) such as Linolenic acid, Stearidonic acid, Eicosapentaenoic acid (as n-3 PUFAs), and Arachidonic acid (as n-6 PUFAs). In addition, various bioactive chemicals, including sterols, are found in lipids (Luo et al., 2015; Pérez et al., 2016; Susanto et al., 2019). These sterols, which are mostly represented by cholesterol and clionasterol, are important bioactive substances with fundamental nutritional and biological qualities such as anticancer, antiobesity, antioxidant, antitumor, antiviral, and are effective in the prevention of cardiovascular disorders, The main nutritional components discovered in seaweeds are fucosterol and isofucosterol (Kendel et al., 2015).

Proteins

The concentration of protein in seaweed varies according to species, seasonal cycle, and seasonal fluctuation factors. It is generally higher for red seaweeds (up to 47% of the dry weight), medium for green seaweeds (35% of the dry weight), and lower for brown seaweeds (24% of the dry weight) (Table 3) (Černá, 2011; Fleurence et al., 2018). Because seaweeds include non-protein nitrogen, their protein content has been overstated, and nitrogen-to-protein conversion ratios smaller than 6.25, often employed for feed components, have been recommended (Makkar et al., 2016). Furthermore, seaweeds include essential amino acids, including glycine, alanine, proline, arginine, glutamic acid, and aspartic acid (Gullón et al., 2020). Phycobiliproteins are of particular interest among algal proteins because, by enzymatic breakdown, peptides with established hypertensive action may be produced by blocking the angiotensin I converting Enzyme (Furuta et al., 2016).

Vitamins

Seaweeds are high in fat-soluble vitamins, including vitamin A, vitamin D, vitamin E, and provitamin A, as well as water-soluble vitamins, including vitamin C, folic acid, pantothenic acid, niacin, riboflavin and vitamin B such as vitamin B12, vitamin B6, vitamin B3, vitamin B2, and vitamin B1 (Hentati et al., 2020). However, some of them only in relatively low content (Škrovánková, 2011) because the vitamin content of seaweeds varies depending on the species. Green seaweeds, for example, had vitamin E concentrations ranging from 8.8-12.0 mg/kg, red seaweeds ranging from 10-26 mg/kg, and brown seaweeds ranging from 1.6-122 mg/kg dried weight (Biancarosa et al., 2018). For vitamin C concentrations, green seaweeds have 0.0347-1.25g/100g, red seaweeds have 0.0353-1.61g/100g, and brown seaweeds 0.0345-1.85g/100g dried weight, and as for essential vitamin B3, green seaweeds range from 0.005–1.0g/100g, red seaweeds range from 0.0951-0.10g/100g and brown seaweeds range from 0.612–0.90g/100g dried weight (Hentati et al., 2018). In addition, the contents of Vitamin B12 in seaweeds also vary. For example, green seaweeds are between 0.06 and 0.786 g/100 g; red seaweeds are between 0.0961 and 1.34g/100g, and brown seaweeds range between 0.0164 and 0.0431g/100g dried weight (Table 4) (Hentati et al., 2018; Cherry et al., 2019).

Minerals

Seaweeds also have a significant concentration of minerals (8-40%), including Na, K, Mg, Fe, and others (Table 5) (Cofrades et al., 2017; Lorenzo et al., 2017). Calcium is the most visible mineral, and it is found in the highest concentration in plant sources. They also contain a lower Na/K ratio than other foods often found in Western diets, which is advantageous for maintaining a healthy cardiovascular system (Circuncisão et al., 2018). In addition, seaweeds contain substantial quantities of iodine, and their consumption can aid in treating iodine deficiency (Zava & Zava, 2011).

Polysaccharides (Hydrocolloids)

Phycocolloids, are hydrocolloids (substances that form a viscous solution when mixed with water) derived from seaweeds. In 2019, the total global import and export of seaweeds and seaweed-based hydrocolloids ($1.74 billion) are estimated at $2.9 billion and $2.65 billion, respectively (FAO, 2021b). Numerous polysaccharides are derived from phycocolloids, including major seaweed polysaccharides like alginate, agar, and carrageenan, which are economically valuable for the pharmaceutical and nutraceutical sectors (Gnanavel et al., 2019). Depending on the species, seaweed polysaccharides range from 4% to 76% by dry weight. Brown seaweeds contain alginates, fucoidans, and laminarin; red seaweeds have carrageenans and agarans; green seaweeds include ulvans. These seaweeds polysaccharides are primarily sulfated (such as agars, carrageenans, fucoidans, and galactans) and non-sulfated (such as alginates and laminaran) polysaccharides that are high in dietary fiber. In addition, they may have prebiotic properties that have been related to the antibacterial, anticancer, anticoagulant, antihyperlipidemic, anti-inflammatory, antiobesity, antioxidant, antiviral, gastroprotective, and immunomodulatory effects (Seong et al., 2019; Gullón et al., 2020; Hentati et al., 2020). Important hydrocolloids, such as agar, carrageenan, and alginates, are also called phytochemicals. They are primarily utilized in human and animal foods, dairy products, confectionery, textiles, paper industries, and in certain other countries, as manure. (Pal et al., 2014).

Red seaweeds (Rhodophyta) such as *Gelidiella* or *Pterocladia, Gelidium,* and *Gracilaria* are sources of agar which composed of two polysaccharides, such as agarose (for gelling) and agaropectin (for thickening). However, agarose is accounting for around 70% of the total in the mixture. Agar also contains hydrophilic galactans consist of α(1-4)-3,6-anhydro-L-galactose, and β9(1-3)-D-galactose (Lee et al., 2017) and it is the first hydrocolloid with the European registration number E406. The Food and Drug Administration (FDA) has designated agar as Generally Recognized as Safe (GRAS) for use as a food additive which approximately 80% of are produced globally. The remaining 10–20% were employed in the pharmaceutical and other biotechnology sectors. Agar may be used in a variety of ways depending on its quality. Low-grade agar is used in foods and industrial applications such as adhesives, casting, impression, paper coating, textile printing dyeing, and other applications. In the medical and pharmaceutical fields, medium grade agar is employed as a gel substrate in biological culture media, anticoagulant agents, bulking agents, capsules, laxatives, and tablets. Finally, highly purified agar, a high-grade agar, is utilized in intermolecular biology separation procedures like electrophoresis, gel chromatography, and immunodiffusion (Pal et al., 2014; Abdul Khalil et al., 2018). On the other hand, carrageenan with European registration name E407 is similar to agar that are derived from red seaweeds but mostly from the species of *Kappaphycus alvarezii, E. denticulata, E. spinosum, B. gelatinae, C. crispus, Gigartina* sp.*, and Hypnea* sp., making up as much as half of the dry weight (Abdul Khalil et al., 2018). It is the general name for a group of naturally occurring water-soluble sulfated galactans with alternate backbones of α(1-4)-3,6-anhydro-D-galactose, and β(1-3)-D-galactose (Subaryono, 2018). There are three basic types of Carrageenan that are commercially classified, these includes iota (ι)-carrageenan, kappa (κ)-carrageenan, lambda (λ)-carrageenan, although other types of carrageenan are also reported such as μ-carrageenan, ν-carrageenan (Rhein-Knudsen et al., 2017). Carrageenan is mainly used in different foods as emulsifiers, thickeners, stabilizers, and protective coating on fresh-cut packaged food. However, since red seaweeds have a variety of species and compositions, this makes the carrageenan as one of the most challenging phycocolloids to characterized. (Abdul Khalil et al., 2018).

Table 5. Mineral content of seaweed (adapted from Circuncisão et al. (2018), Martínez–Hernández et al. (2018), Olsson et al. (2020))

Species	Mineral contents										
	Na	*Cl*	*K*	*S*	*Mg*	*Ca*	*P*	*Br*	*Sr*	*I*	*Cr*	*Pb*
Green seaweeds: *Ulva* sp.	10.8[a]	28.5[a]	19.5[a]	50.7[a]	33[a]	9.1[a]	1.1[a]	451.4[a]	101.9[a]	69[a]	-	-
Brown seaweeds: *Laminariia ochroleuca, U. pinnatifida, H. elongata*	40.2-113[a]	91.6-84.3[a]	84.3-3.7[a]	12.6-9.1[a]	7.6-4.1[a]	11.2-10.6[a]	5.4-1.2[a]	847-364[a]	789.1-629.7[a]	5552-96[a]	-	-
Red seaweeds: *Palmaria palmata, C. crispus, Porphyra* sp.	77.5-22.2[a]	91.9-23.7[a]	76.2-31.9[a]	53-5.6[a]	7.5-2.3[a]	22.3-4.3[a]	3.2-1.6[a]	1191-359.3[a]	167.5-43.8[a]	472-76[a]	-	-
Green seaweeds: *Cladophora rupestris, Cladophora* sp., *U. intestinalis, U. lactuca*	73-31[b]	-	85-17[b]	-	26.6-4.4[b]	10.4-5.4[b]	171-0.97[b]	-	-	-	19.4-0.5[a]	4.32-0.11[a]
Brown seaweeds: *Ascophyllum nodosum, C. filum, D. aculeate, F. serratus, F. vesiculosus, H. siliquosa, L. digitata, S. latissima, S. cirrosa*	51-25[b]	-	84-15[b]	-	8.9-4.6[b]	47.9-9.5[b]	2.16-0.67[b]	-	-	-	2.8-0.2[a]	10.0-0.05[a]
Red seaweeds: *Ahnfeltia. plicata, B. byssoides, Ceramium sp., C. crispus, C. purpureum, D. sanguinea, D. carnosa, F. lumbricalis, R. confervoides*	52-25[b]	-	58-22[b]	-	15.1-5.1[b]	82.2-3.2[b]	2.42-1.03[b]	-	-	-	4.0-0.3[a]	7.24-0.08[a]
Green seaweeds: *Ulva* sp.	24.0-11[b]	-	26.0-12[b]	-	38-15[b]	20-3.7[b]	0.5-7[b]	-	-	130-8[a]	-	-
Brown seaweeds: *Ascophyllum nodosum, A. esculenta, F. spiralis, F. vesiculosus, H. elongata, Laminaria* sp., *S. latissima, U. pinnatifida*	71-6[b]	-	120-8[b]	-	12.0-2[b]	31.0-1[b]	4-0.5[b]	-	-	9014-17[a]	-	-
Red seaweeds: *Chondrus crispus, Gracilaria sp., P. calcareum, P. palmata, Porphyra sp.*	44-3[b]	-	96-1[b]	-	9.0-1[b]	303-0.4[b]	6-0.6[b]	-	-	260-34[a]	-	-

Table 5 continued

Species	Mineral contents											
	Si	*Fe*	*Al*	*Mn*	*Zr*	*Ti*	*Cu*	*Zn*	*Ni*	*As*	*Cd*	*Co*
Green seaweeds: *Ulva* sp.	1390[a]	273.5[a]	407[a]	17[a]	57.5[a]	18[a]	10[a]	60.2[a]	-	-	-	-
Brown seaweeds: *Laminariia ochroleuca, U. pinnatifida, H. elongata*	930.5-180[a]	229-87[a]	375-59[a]	73-20[a]	36-32[a]	228-24[a]	18.5-10.5[a]	243-42.4[a]	-	-	-	-
Red seaweeds: *Palmaria palmata, C. crispus, Porphyra* sp.	1120-546.5[a]	205.5-123.5[a]	360-178.5[a]	33.5-26[a]	45.5-40[a]	29.5[a]	15.5-14[a]	53.9-22.6[a]	-	-	-	-
Green seaweeds: *Cladophora rupestris, Cladophora* sp., *U. intestinalis, U. lactuca*	1.93-0.31[a]	452-89[a]	499-64[a]	418-19[a]	-	-	97-4.6[a]	22.0-6.0[a]	7.0-1.5[a]	117-3.7[a]	0.73-0.06[a]	1.44-0.09[a]
Brown seaweeds: *Ascophyllum nodosum, C. filum, D. aculeate, F. serratus, F. vesiculosus, H. siliquosa, L. digitata, S. latissima, S. cirrosa*	4.42-<0.1[a]	910-24[a]	1130-4[a]	237-3[a]	-	-	16.4-1.1[a]	68-14[a]	18.3-0.5[a]	58.9-19.6[a]	1.46-0.06[a]	1.63-0.08[a]
Red seaweeds: *Ahnfeltia. plicata, B. byssoides, Ceramium sp., C. crispus, C. purpureum, D. sanguinea, D. carnosa, F. lumbricalis, R. confervoides*	5.77-<0.1[a]	1710-59.3[a]	2060-10[a]	1820-18[a]	-	-	13.4-3.0[a]	254-20[a]	11-3.2[a]	35.8-6.2[a]	7.76-0.12[a]	4.91-0.20[a]
Green seaweeds: *Ulva* sp.	-	6000-139[a]	-	637-13[a]	-	-	33-2[a]	64-4[a]	-	-	-	1.4-0.2[a]
Brown seaweeds: *Ascophyllum nodosum A. esculenta, F. spiralis, F. vesiculosus, H. elongata, Laminaria* sp., *S. latissima, U. pinnatifida*	-	1854-4[a]	-	547-1[a]	-	-	80-0.3[a]	740-2[a]	-	-	-	5-0.01[a]
Red seaweeds: *Chondrus crispus, Gracilaria sp., P. calcareum, P. palmata, Porphyra sp.*	-	2110-35[a]	-	653-2[a]	-	-	35-1[a]	95-5[a]	-	-	-	7-0.03[a]

Note: [a] μg/kg, [b] g/kg

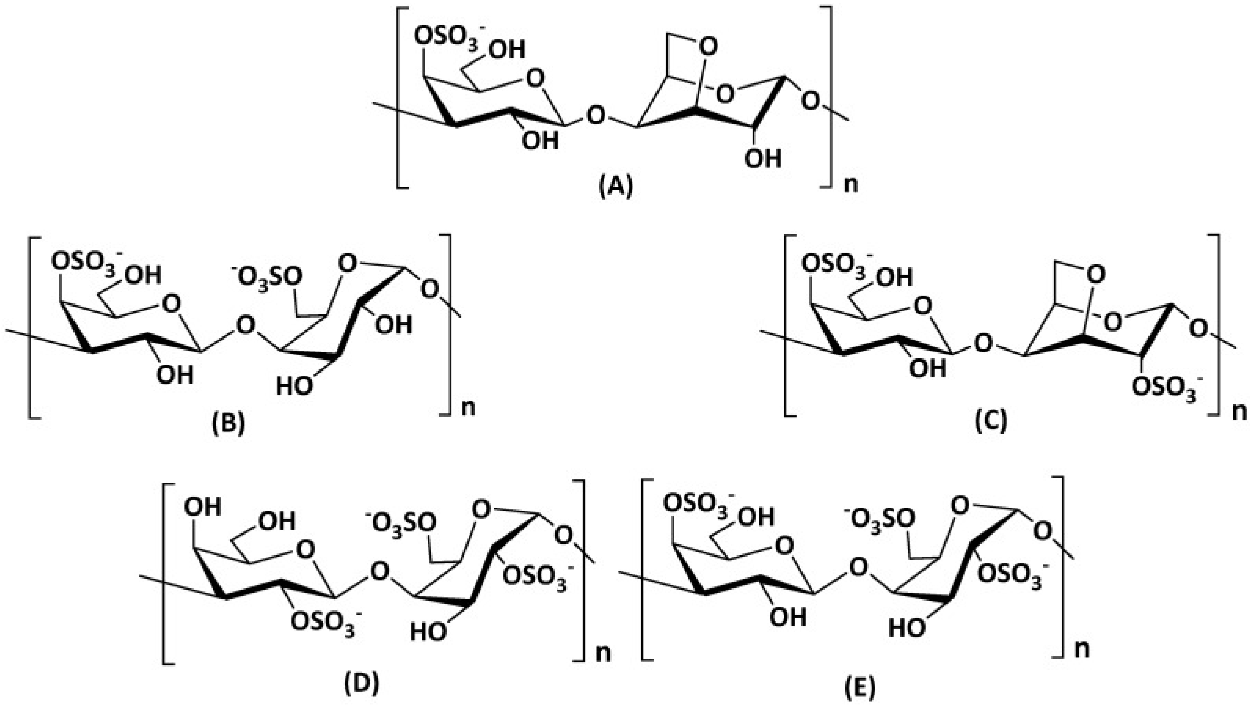

Figure 3. Structure of different types of carrageenans. (A) κ-carrageenan, (B) μ-carrageenan, (C) ι-carrageenan, (D) λ-carrageenan and (E) ν-carrageenan (adapted from Nešić et al., 2019)

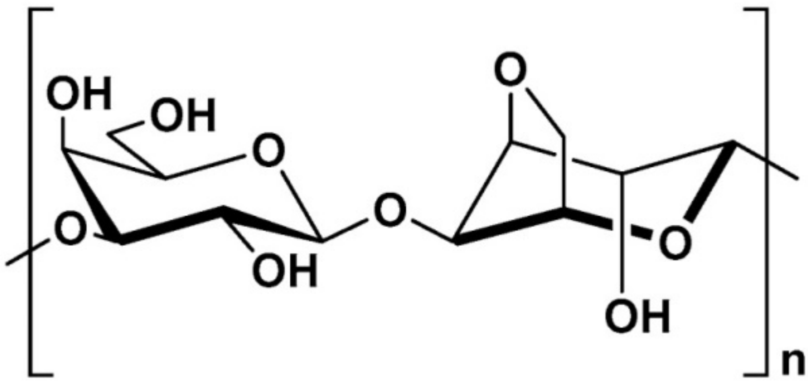

Figure 4. Structure of agar (adapted from Nešić et al., 2019)

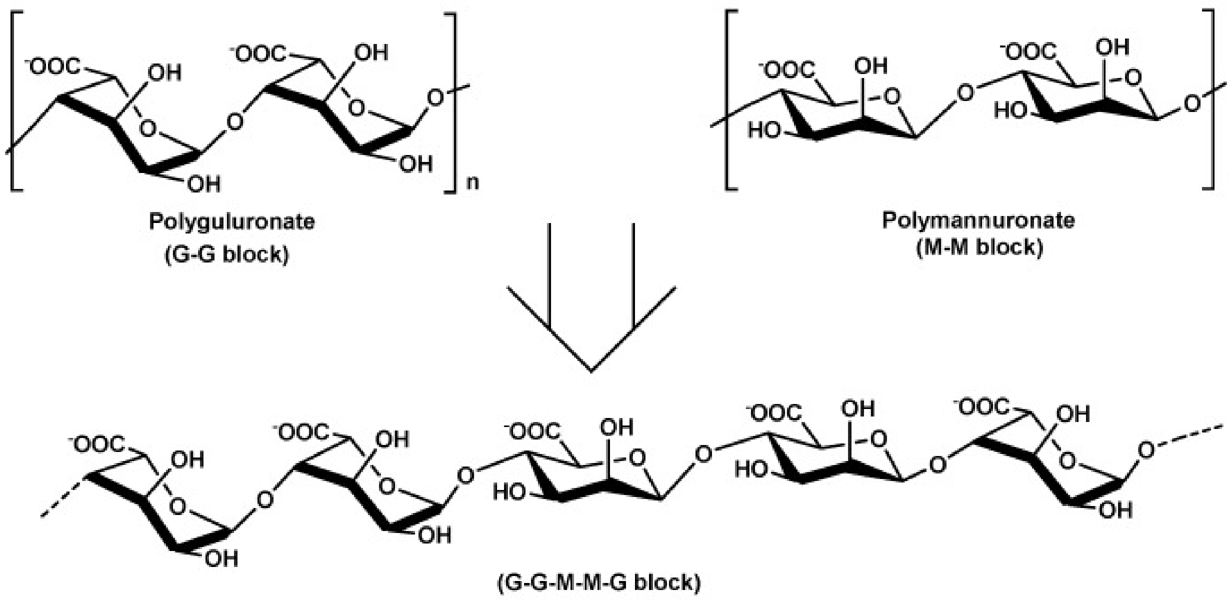

Figure 5. Structure of alginate (glycosidic bond conformations of β-d-mannuronic acid and α-l-guluronic acid) (adapted from Nešić et al., 2019)

Alginates, with a European registration number of E401 to E405 are derived from brown seaweeds such as *Ascophyllum nodosum, E. maxima, L. digitata, L. hyperborean, L. japonica, L. nigrescens, M. pyrifera, Sargassum* sp. and other brown seaweeds. Alginates are copolymers of α(1–4) linked α-L-galactouronic and β-D-mannouronic acids (Ramnani et al., 2012; Abdul Khalil et al., 2018). It is mainly utilized in the food and pharmaceutical industries due to binding metal ions and generating viscous solutions. It is used as gelling agents and also as sizing agent for cotton yarn in the textile sectors. Alginate comes in two types: acid and salt. The asalginic acid is the acid type, it is a linear polyuronic acid, whereas the salt type is an important component in the cell wall of brown seaweeds, accounting from 40 to 47% of the algal biomass by dry weight (Pal et al., 2014; Abdul Khalil et al., 2018).

Potential risk factors of seaweeds

Seaweeds, well-known for their health advantages and high concentrations of essential components, might represent a health danger by absorbing high quantities of heavy metals and Iodine from the environment (Filippini et al., 2021).

Exposure to Heavy metals

There are approximately 145 species of seaweeds that are consumed globally in amounts as high as 97,000 tons annually in some countries such as Japan (Cheney, 2016). *Porphyra* sp. is one of the famous edible seaweed in Southeast Asia and around the world. In Japan, it is known as "nori" and is eaten as nori sheets with the Japanese delicacy "sushi." It is also known as "kim" in Korea, "zicai" in China, "purple laver" in the Britain and Ireland, "karengo" in New Zealand, and "Laver" in the United States, United Kingdom, and Canada (Baweja et al., 2016). According to Rubio et al. (2017), red seaweeds, particularly the *Porphyra* species, have more trace and dangerous elements. As a result, the average cadmium (Cd) level in conventional farming is two points higher (0.28 mg/kg) than in organic cultivation (0.13 mg/kg). Furthermore, 4g per day of seaweeds intake helps increase the dietary intake of metals like magnesium (Mg) and chromium (Cr). In addition, the average aluminum (Al), cadmium (Cd) and lead (Pb) daily intakes were 0.064, 0.001, and 0.0003 mg/day, respectively. According to the research, exposure to these toxic metals (Al, Cd, and Pb) did not pose serious health risks. But, other hazardous metals should also be monitored as per recommendation of the author (Rubio et al., 2017).

Exposure to Arsenic

Another critical health concern connected with seaweed is arsenic. Arsenic species can be harmful (inorganic arsenic, a carcinogen of class I), innocuous (arsenobetaine), or potentially dangerous (fat-soluble arsenic, arsenosugars, and other organoarsenic). The International Agency for Research on Cancer has identified inorganic arsenic as a human carcinogen capable of causing bladder, skin, and lung cancer. Arsenic use has also been related to an increased risk of heart diseases and diabetes (Murai et al., 2021). Arsenic is naturally accumulated in seaweeds, particularly Hijiki, an edible brown seaweed (Flora, 2015) that is harvested and consumed in Japan. It is sun dried, boiled, then dried again until it is completely black. It is frequently used as a topping for cooked rice or as a breakfast condiment. However, because of the high levels of arsenic, people were warned not to consumed too much of it (Mouritsen et al., 2018). This wet Hijiki has 0.22 mg of inorganic arsenic per serving (20g), accounting for 80% of the arsenic content (Yokoi & Konomi, 2012) that exceeds the World Health Organization's recommended daily intake standards. However, there are no evidence of the health consequences of arsenic poisoning induced by the inorganic arsenic from Hijiki has been shown. It has been claimed that adverse effects on people's health are rare unless they consume vast amounts of Hijiki (Murai et al., 2021).

High Iodine Concentrations

Excessive iodine content is another potential concern of seaweeds (Bouga & Combet, 2015). Seaweed absorbs iodine from seawater and is hence an excellent source of iodine in the diet. Eating enough seaweeds may help to eliminate iodine deficiency. Too much iodine, on the other hand, is hazardous to one's health (Yeh et al., 2014). Kombu (*S. japonica*), wakame (*U. pinnatifida*), and nori (*Porphyra* sp.) are edible seaweeds that are commonly consumed in Asia and have a high concentration of iodine. According to Yeh et al. (2014), the highest average iodine content is found in kelp (*S. japonica*), commonly known as Kombu in Japan, with 2523.5 mg/kg, followed by wakame (139.7 mg/kg) and nori (36.9 mg/kg). Kombu is a popular food and dietary supplement, notably in Japan. However, research suggests that kombu contains a high iodine concentration, implying that consuming too much kelp/kombu supplements on a daily basis for an extended period of time may represent a risk to consumers, such as thyroid dysfunction or hyperthyroidism (Dasgupta & Wahed, 2014). Iodine levels in seaweed can be extremely high and even hazardous. Surprisingly, Japanese people's high iodine consumption is regarded as one of the reasons they are among the healthiest people on the planet. In Japan, however, the average daily consumption of Iodine is estimated to be 1,000–3,000 µg (667–2,000% of the RDI). This poses a concern to people who consume seaweed daily, as the tolerable upper limit (TUL) for adults is 600 g/d (EFSA) and 1100 g/d (World Health Organization) (Zava & Zava, 2011; Cherry et al., 2019).

Furthermore, several studies have found a link between excessive iodine intake and illnesses such as hypo- and hyperthyroidism, goiter, and thyroiditis, whereas, iodine deficiency causes hypothyroidism (Combet et al., 2014; Desideri et al., 2016; Aakre et al., 2020). Many of the symptoms of hyperthyroidism may differ from different individual person. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), common hyperthyroidism symptoms include fatigue or muscle weakness, diarrhea, hand tremors, heat intolerance, mood swings, nervousness or irritability, rapid and irregular heartbeat, sleeping difficulties, weight loss, and goiter (an enlarged thyroid that causes the neck to appear bloated and can obstruct regular breathing and swallowing) (NIDDK, 2016). However, the epidemiological research describing the risks and benefits of consuming iodine from seaweeds is inconclusive (Cherry et al., 2019).

Uses of Seaweeds

As previously indicated, seaweeds have a high concentration of bioactive compounds. Previously, seaweed was mainly used as a vegetable and consumed as raw. It is also an excellent source of gelling and thickening ingredients in food for humans and animals (Hentati et al., 2020). A new study has shown its potential for alternative medicine in recent years. Antibacterial (Moubayed et al., 2017), anticancer (Haq et al., 2019), anticoagulant (Liu et al., 2018), antidiabetic (Gunathilaka et al., 2020), antiestrogenic (Teas et al., 2013), antifungal (De Corato et al., 2017), antihyperlipidemic (Yim et al., 2019), antimycotic (Saito & Lal, 2019), antihypertensive (Seca & Pinto, 2018), anti-inflammatory (Saraswati et al., 2019), antiobesity (Sun et al., 2018), antioxidant (Hermund, 2018), antiviral (Gheda et al., 2016), immunomodulatory (Palstra et al., 2018), neuroprotective (Silva et al., 2018), tissue healing (Premarathna et al., 2019b) and thyroid-stimulating properties have been demonstrated in red, brown, and green seaweeds (Khalid et al., 2018). Furthermore, seaweeds are used in the cosmetic and pharmaceutical industries (Hentati et al., 2020). They are now a possible energy source as biofuels (Del Río et al., 2020) and important as biobased goods (Nakhate & van der Meer, 2021) and biopolymers (Jumaidin et al., 2018). In addition, Seaweeds are also employed in aquaculture as probiotics (Vatsos & Rebours, 2015; Nazarudin et al., 2020) animal feed additive (Makkar et al., 2016; Morais et al., 2020), water purifier (Arumugam et al., 2018), bio-elicitors (Agarwal et al., 2021), plant fertilizer (Ruban & Govindasamy, 2018), biostimulants (Pereira & Cotas, 2019) and as seaweed-based liquid oganic fertilizer to other seaweed such as to stimulate the growth and quality of *G. verrucosa* (Nasmia et al., 2020).

Extraction of Seaweeds

To extract novel metabolites from seaweed without causing degradation, modern techniques such as Enzyme Assisted Extraction (EAE), Microwave Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), Supercritical Fluid Extraction (SFE), and Pressure Solvent Extraction (PSE) have been employed (Table 6) due to its advantages over the traditional techniques. However, to generate extracts containing the necessary bioactive components, the process parameters of each technique must be modified (Cikoš et al., 2018).

Table 6 shows the seaweed extraction process (common bioactive compounds extracted, advantages and limitations of green extraction method). Table 6 also shows the differences, advantages and limitations of the modern (green) techniques. EAE has advantages for industrial applications since it can be scaled up, it has a high catalytic efficiency and specificity, and it is a safe method of extraction because the enzymes utilized are food grade level. However, the full benefits of this extraction process can only be realized if the limitations such as expensive cost, lengthy extraction time that can range from hours to days, lack of substrate specific enzyme availability, and enzymatic hydrolysis efficacy are overcome. UAE on the other hand, is an extraction approach that has been employed in the industrial extraction of bioactive chemicals from many natural resources, and it has recently been shown that it can also be used in the extraction of new metabolites from seaweeds. For SFE, this approach is quite expensive for the extraction due to the high pressure equipment requirements, but it can also be utilized for the extraction of new metabolites from seaweeds. In fact, it is a very promising method of extraction because it produces extracts with great purity and no residues. MAE and PLE, on the other hand, are quite risky because they require high

Table 6. Seaweed extraction process (adapted from Admassu et al. (2018), Kadam et al. (2015), Praveen et al. (2019))

Extraction Method	Procedure	Bioactive Components	Advantages	Limits
Enzyme-Assisted Extraction (EAE)	*Incorporating food-grade enzymes such as *cellulase, a-amylase, pepsin, viscozyme, cellucast, termamyl, ultraflo, carrageenase, agarase, xylanase, kojizyme, neutrase, alcalase, and umamizyme* into seaweeds. * Degradation of glycosidic bonds and other internal bonds	*Fucoxanthin *Lipids *Phlorotannins *Phenolic compounds	*Time efficiency *High catalytic efficiency & specificity *Enzymes employed are eco-friendly, non-toxic, and food grade level *High yield *High possibility for industrial scale-up	*Slow extraction procedure (takes hours to days) *Enzymatic hydrolysis effectiveness is very low if the material's moisture content is very low *Limited owing to its costly price
Microwave-Assisted Extraction (MAE)	*Most researched extraction technique. *Microwave energy was used to heat solvent-containing samples. *Dielectric and total volumetric heating by microwaves. *2.45 GHz	*Sulfated polysaccharides, such as fucoidan, ulvan, and rhamnan sulfate.	*Short treatment time *Can utilize organic solvents and water *Ideal for thermally labile chemicals *Better than traditional and Soxhlet methods	*The only solvent that can be used is one with a high dielectric constant, and a low dissipation factor *High capital cost *Potential explosion danger, especially with MAE closed vessels
Ultrasound-Assisted Extraction (UAE)	*Ultrasonic radiation pressure was used to generate intense mixing and agitation, which promotes Extraction. *Compression and rarefaction (pressure variation and cavitation) *20 kHz *50-60 kHz	*Polyphenols * Fucose and uronic acid * Laminarin *Phycobiliary proteins *Taurine *Antioxidants	*Short treatment time *Less solvent usage *High extraction yields *Low cost	*Extraction efficiency varies according to plant matrix *Solvents with low surface tension, viscosity, and vapor pressure are preferred *Excessive sonication may degrade extract quality
Supercritical Fluid Extraction (SFE) Pressurized	*The supercritical fluid's temperature and pressure are both greater than the critical point.	*Fatty acids (ω-3) *Carotenoids, * Fucoxanthin, *Fluorotannins * Volatile compounds *Polyphenols, *Cytokinins, * Auxins, Microelements, and macro elements	*Technology for green Extraction *Extracts with excellent purities and no residues *Extracts made without the use of solvents *Extraction of thermolabile compounds requires a short extraction period.	*Expensive high-pressure equipment is required. *Polar chemicals may be difficult to remove. *Processing costs and energy usage are both high.
Pressurized Solvent Extraction (PSE)/ Pressurized Liquid Extraction (PLE) Reaction	*To extract analytes, a relative amount of solvent (toluene, hexane, or acetone) was used at high temperatures and pressure.	*Polyphenols *Fluorotannins *Neo antioxidants, *Amino acids *Polysaccharides *Fucoidan *Total organic carbon *Minerals	*Green extraction method (water can be used as solvents) *Extraction efficiency is high, using fewer solvents. *Extraction time is limited.	*The cost of the required high-pressure equipment is too expensive. *High-temperature extraction may cause thermolabile compounds to degrade.

Table 7. Extraction method of different species of seaweeds

Extraction Method	Species	References
Enzyme-Assisted Extraction (EAE)	*Macrocystis pyrifera, C. chamissoi, S. boveanum, S. anguistifolium, F. irregularis, P. palmata, C. crispus, C. fragile, S. muticum, O. pinnatifida, C. tomentosum, U. armoricana, S. ilicifolium, S. polycystum*	Kulshreshtha et al., 2015; Rodrigues et al., 2015; Hardouin et al., 2016; Naseri et al., 2020; Vásquez et al., 2019; Sabeena et al., 2020
Microwave-Assisted Extraction (MAE)	*Gelidiella. acerosa, U. pinnatifida, S. fusiformis, L. japonica, F. vesiculosus, G. vermiculophylla, G. racemosa, E. prolifera, F. virsoides, C. barbata, E. radiata*	Rodriguez-Jasso et al., 2011; Michalak et al., 2015; Charoensiddhi et al., 2015; Singh et al., 2017; Dobrinčić et al., 2021
Ultrasound-Assisted Extraction (UAE)	*Ascophyllum nodosum, S. muticum, O. pinnatifida, C. tomentosum, S.binderi , T. ornate, K. alvarezii, E. denticulatum, S. mcclurei, G. turuturu*	Cecile et al., 2015; Kadam et al., 2015; Rodrigues et al., 2015; Youssouf et al., 2017; Thao My et al., 2020
Supercritical Fluid Extraction (SFE)	*Ulva flexuosa, D. membranacea D. lingulatus, S. hemiphyllum, L. vadosa, S. muticum, U. pinnatifida, G. tenax, Z. marina, P. oceana, C. glomerata, U. clathrata, P. fucoides, G. mammillaris*	Zheng et al., 2012; Quitain et al., 2013; Pérez-López et al., 2014; Becerra et al., 2015; Michalak et al., 2016; Machmudah et al., 2018; Sevimli-Gur & Yesil-Celiktas, 2019
Pressurized Solvent Extraction (PSE)	*Ulva intestinalis, U. lactuca, F. vesiculosus, D. dichotoma, C. baccata, H. elongate, S. japonica, S. muticum*	Sánchez-Camargo et al., 2017; Otero et al., 2018

temperatures. Thermolabile chemicals may deteriorate as a result of high-temperature extraction (Kadam et al., 2015; Admassu et al., 2018; Cikoš et al., 2018; Praveen et al., 2019). Table 7 shows the different species of seaweeds that has been extracted using these novel green methods of extraction.

Purification of seaweed extracts

Given that every biological matrix, including seaweeds, is a complicated combination of different macromolecules and micromolecules, it is critical to eliminate all extra materials while retaining those containing the biomolecules of interest. As a result, it is vital to use analytical methods to characterize these bioactive chemicals. This is usually achieved by first preparing the sample and then extracting it by various methods using the required solvents and within each phase of the procedure, the necessary number of substeps is determined by the matrix's complexity and the type of target biomolecule under investigation. Finally, the extracted bioactive chemicals are subjected to various types of chromatography as well as identification procedures such as mass spectrometry (Misra et al., 2015; Batool & Menaa, 2020).

Conclusion

Seaweeds, are regarded as an economically important biological resource because they contain a diverse range of bioactive compounds, such as different pigments, phenolic compounds, lipids, vitamins, proteins, minerals, polysaccharides, polyunsaturated fatty acids, and other essential bioactive compounds with a wide range of biological functions. Seaweeds are commercially sold as fresh and extracts, mainly in the food industry, pharmaceutical industry, cosmetics industry, and many other industries. Although seaweeds are generally healthy and safe, they may still pose significant risks, notably high iodine concentration, heavy metals, and arsenic exposure. However, there is still a lacked of information on this manner because the epidemiological study is still inconclusive. Furthermore, various green extraction techniques were employed to isolate bioactive components, then purified using chromatographic analysis to confirm the extract's purity.

Compliance With Ethical Standards

Authors' Contributions

The study was conceptualized by MQA and SY, and MQA wrote the original draft of the article. SY then edited the manuscript for additional corrections. Finally, the authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Aakre, I., Evensen, L. T., Kjellevold, M., Dahl, L., Henjum, S., Alexander, J., Madsen, L., & Markhus, M. W. (2020). Iodine status and thyroid function in a group of seaweed consumers in norway. *Nutrients*, *12*(11), 1–14. https://doi.org/10.3390/nu12113483

Abdul Khalil, H. P. S., Lai, T. K., Tye, Y. Y., Rizal, S., Chong, E. W. N., Yap, S. W., Hamzah, A. A., Nurul Fazita, M. R., & Paridah, M. T. (2018). A review of extractions of seaweed hydrocolloids: Properties and applications. *Express Polymer Letters*, *12*(4), 296–317. https://doi.org/10.3144/expresspolymlett.2018.27

Admassu, H., Gasmalla, M. A. A., Yang, R., & Zhao, W. (2018). Bioactive peptides derived from seaweed protein and their health benefits: antihypertensive, antioxidant, and antidiabetic properties. *Journal of Food Science*, *83*(1), 6–16. https://doi.org/10.1111/1750-3841.14011

Agarwal, P. K., Dangariya, M., & Agarwal, P. (2021). Seaweed extracts: Potential biodegradable, environmentally friendly resources for regulating plant defence. *Algal Research*, *58*(October 2020), 102363. https://doi.org/10.1016/j.algal.2021.102363

Arumugam, N., Chelliapan, S., Kamyab, H., Thirugnana, S., Othman, N., & Nasri, N. S. (2018). Treatment of wastewater using seaweed: A review. *International Journal of Environmental Research and Public Health*, *15*(12). 2851. https://doi.org/10.3390/ijerph15122851

Aryee, A. N., Agyei, D., & Akanbi, T. O. (2018). Recovery and utilization of seaweed pigments in food processing. *Current Opinion in Food Science*, *19*, 113–119. https://doi.org/10.1016/j.cofs.2018.03.013

Ashraf, M. T., Fang, C., Bochenski, T., Cybulska, I., Alassali, A., Sowunmi, A., Farzanah, R., Brudecki, G. P., Chaturvedi, T., Haris, S., Schmidt, J. E., & Thomsen, M. H. (2016). Estimation of bioenergy potential for local biomass in the United Arab Emirates. *Emirates Journal of Food and Agriculture*, *28*(2), 99–106. https://doi.org/10.9755/ejfa.2015-04-060

Baleta, F. N., & Nalleb, J. P. (2016). Species composition, abundance and diversity of seaweeds along the intertidal zone of Nangaramoan, San Vicente, Sta. Ana, Cagayan, Philippines. *AACL Bioflux*, *9*(2), 250–259.

Batool, A., & Menaa, F. (2020). Concentration and purification of seaweed components by chromatography methods. In Torres, M. D., Kraan, S., & Dominguez, H. (Eds.), *Sustainable Seaweed Technologies: Cultivation, Biorefinery, and Applications* (pp. 315-370). Elsevier. https://doi.org/10.1016/b978-0-12-817943-7.00013-5

Baweja, P., Kumar, S., Sahoo, D., & Levine, I. (2016). Biology of seaweeds. In Fleurence, J., & Ira Levine, I. (Eds.), *Seaweed in Health and Disease Prevention* (pp. 41-106). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802772-1.00003-8

Becerra, M., Boutefnouchet, S., Córdoba, O., Vitorino, G. P., Brehu, L., Lamour, I., Laimay, F., Efstathiou, A., Smirlis, D., Michel, S., Kritsanida, M., Flores, M. L., & Grougnet, R. (2015). Antileishmanial activity of fucosterol recovered from Lessonia vadosa Searles (Lessoniaceae) by SFE, PSE and CPC. *Phytochemistry Letters*, *11*, 418–423. https://doi.org/10.1016/j.phytol.2014.12.019

Bedoux, G., Hardouin, K., Burlot, A. S., & Bourgougnon, N. (2014). Bioactive components from seaweeds: Cosmetic applications and future development. In Bourgougnon, E. (Ed.), *Advances in Botanical Research Volume 71* (pp. 345-378). Elsevier. https://doi.org/10.1016/B978-0-12-408062-1.00012-3

Biancarosa, I., Belghit, I., Bruckner, C. G., Liland, N. S., Waagbø, R., Amlund, H., Heesch, S., & Lock, E. J. (2018). Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and feed. *Journal of the Science of Food and Agriculture*, *98*(5), 2035–2042. https://doi.org/10.1002/jsfa.8798

Bouga, M., & Combet, E. (2015). Emergence of seaweed and seaweed-containing foods in the uk: Focus on labeling, iodine content, toxicity and nutrition. *Foods*, *4*(2), 240–253. https://doi.org/10.3390/foods4020240

Cecile, L. G., Justine, D., Claire, D.-M., Sandrine, B., Jean-Yves, R., Joel, F., & Jean-Pascal, B. (2015). Achimer Ultrasound-assisted extraction of R-phycoerythrin from Grateloupia turuturu with and without enzyme addition. *Algal Research*, *12*(November), 522–528.

Černá, M. (2011). Seaweed proteins and amino acids as nutraceuticals. In Kim, S. -K.(Ed.), *Advances in Food and Nutrition Research Volume 64* (pp. 297-312) (1st ed., Vol. 64). Elsevier Inc. https://doi.org/10.1016/B978-0-12-387669-0.00024-7

Charoensiddhi, S., Franco, C., Su, P., & Zhang, W. (2015). Improved antioxidant activities of brown seaweed Ecklonia radiata extracts prepared by microwave-assisted enzymatic extraction. *Journal of Applied Phycology*, *27*(5), 2049–2058. https://doi.org/10.1007/s10811-014-0476-2

Charrier, B., Abreu, M. H., Araujo, R., Bruhn, A., Coates, J. C., De Clerck, O., Katsaros, C., Robaina, R. R., & Wichard, T. (2017). Furthering knowledge of seaweed growth and development to facilitate sustainable aquaculture. *New Phytologist*, *216*(4), 967–975. https://doi.org/10.1111/nph.14728

Chen, K., Ríos, J. J., Pérez-Gálvez, A., & Roca, M. (2017). Comprehensive chlorophyll composition in the main edible seaweeds. *Food Chemistry*, *228*(February), 625–633. https://doi.org/10.1016/j.foodchem.2017.02.036

Cheney, D. (2016). Toxic and Harmful Seaweeds. In Fleurence, J., & Levine, I. (Eds.), *Seaweed in Health and Disease Prevention* (pp. 407-421). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802772-1.00013-0

Cherry, P., O'hara, C., Magee, P. J., Mcsorley, E. M., & Allsopp, P. J. (2019). Risks and benefits of consuming edible seaweeds. *Nutrition Reviews*, *77*(5), 307–329. https://doi.org/10.1093/nutrit/nuy066

Chopin, T., & Tacon, A. G. J. (2021). Importance of Seaweeds and Extractive Species in Global Aquaculture Production. *Reviews in Fisheries Science and Aquaculture*, *29*(2), 139–148. https://doi.org/10.1080/23308249.2020.1810626

Cikoš, A. M., Jokić, S., Šubarić, D., & Jerković, I. (2018). Overview on the application of modern methods for the extraction of bioactive compounds from marine macroalgae. *Marine Drugs*, *16*(10), 348. https://doi.org/10.3390/md16100348

Circuncisão, A. R., Catarino, M. D., Cardoso, S. M., & Silva, A. M. S. (2018). Minerals from macroalgae origin: Health benefits and risks for consumers. *Marine Drugs*, *16*(11), 400. https://doi.org/10.3390/md16110400

Cofrades, S., Benedí, J., Garcimartin, A., Sánchez-Muniz, F. J., & Jimenez-Colmenero, F. (2017). A comprehensive approach to formulation of seaweed-enriched meat products: From technological development to assessment of healthy properties. *Food Research International*, *99*, 1084–1094. https://doi.org/10.1016/j.foodres.2016.06.029

Collins, K. G., Fitzgerald, G. F., Stanton, C., & Ross, R. P. (2016). Looking beyond the terrestrial: The potential of seaweed derived bioactives to treat non-communicable diseases. *Marine Drugs*, *14*(3), 1–31. https://doi.org/10.3390/md14030060

Combet, E., Ma, Z. F., Cousins, F., Thompson, B., & Lean, M. E. J. (2014). Low-level seaweed supplementation improves iodine status in iodine-insufficient women. *British Journal of Nutrition*, *112*(5), 753–761. https://doi.org/10.1017/S0007114514001573

Cotas, J., Pacheco, D., Gonçalves, A.M., Silva, P., Carvalho, L.G., & Pereira, L. (2021). Seaweeds' nutraceutical and biomedical potential in cancer therapy: a concise review. *Journal of Cancer Metastasis and Treatment*, *7*, 13. https://doi.org/10.20517/2394-4722.2020.134

Dasgupta, A., & Wahed, A. (2014). Effect of Herbal Supplements on Clinical Laboratory Test Results. In Dasgupta, A., & Wahed, A. (Eds.), *Clinical Chemistry, Immunology and Laboratory Quality Control: A Comprehensive Review for Board Preparation, Certification and Clinical Practice* (pp. 449–459). Elsevier. https://doi.org/10.1016/b978-0-12-407821-5.00025-5

De Corato, U., Salimbeni, R., De Pretis, A., Avella, N., & Patruno, G. (2017). Antifungal activity of crude extracts from brown and red seaweeds by a supercritical carbon dioxide technique against fruit postharvest fungal diseases. *Postharvest Biology and Technology*, *131*, 16–30. https://doi.org/10.1016/j.postharvbio.2017.04.011

De San, M. (2012). The farming of seaweeds. FAO/IOC. 29p.

Del Río, P. G., Gomes-Dias, J. S., Rocha, C. M. R., Romaní, A., Garrote, G., & Domingues, L. (2020). Recent trends on seaweed fractionation for liquid biofuels production. *Bioresource Technology*, *299*(October 2019), 122613. https://doi.org/10.1016/j.biortech.2019.122613

Desideri, D., Cantaluppi, C., Ceccotto, F., Meli, M. A., Roselli, C., & Feduzi, L. (2016). Essential and toxic elements in seaweeds for human consumption. *Journal of Toxicology and Environmental Health-Part A: Current Issues*, *79*(3), 112–122. https://doi.org/10.1080/15287394.2015.1113598

Dobrinčić, A., Pedisić, S., Zorić, Z., Jurin, M., Roje, M., Čož-Rakovac, R., & Dragović-Uzelac, V. (2021). Microwave assisted extraction and pressurized liquid extraction of sulfated polysaccharides from fucus virsoides and *Cystoseira barbata*. *Foods*, *10*(7), 1481. https://doi.org/10.3390/foods10071481

FAO. (2020). Food and Agriculture Organization of United States. The State of World Fisheries and Aquaculture 2020. Sustainability in action. In FAO. https://doi.org/https://doi.org/10.4060/ca9229en

FAO. (2021a). Food and Agriculture Organization of United States. Global seaweeds and microalgae production, 1950 – 2019. Food and Agricultural Organization of the United Nations. Rome, June, 2021. pp.5. https://www.fao.org/fishery/en/publications/280709

FAO. (2021b). Food and Agriculture Organization of United States. Global status of seaweed production, trade and utilization. Seaweed Innovation Forum Belize, May, 28th.

Filippini, M., Baldisserotto, A., Menotta, S., Fedrizzi, G., Rubini, S., Gigliotti, D., Valpiani, G., Buzzi, R., Manfredini, S., & Vertuani, S. (2021). Heavy metals and potential risks in edible seaweed on the market in Italy. *Chemosphere*, *263*, 127983. https://doi.org/10.1016/j.chemosphere.2020.127983

Fleurence, J. (2016). Seaweeds as food. In Fleurence, J., & Ira Levine, I. (Eds.), *Seaweed in Health and Disease Prevention* (pp. 149-167). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802772-1.00005-1

Fleurence, J., Morançais, M., & Dumay, J. (2018). Seaweed proteins. In Yada, R. Y. (Ed.), *Proteins in Food Processing (Second Edition)* (pp. 245-262). Elsevier. https://doi.org/10.1016/B978-0-08-100722-8.00010-3

Flora, S. J. S. (2015). Arsenic: Chemistry, occurrence, and exposure. In Flora, S. J. S. (Ed.), *Handbook of Arsenic Toxicology* (pp. 1-49). https://doi.org/10.1016/B978-0-12-418688-0.00001-0

Furuta, T., Miyabe, Y., Yasui, H., Kinoshita, Y., & Kishimura, H. (2016). Angiotensin I converting enzyme inhibitory peptides derived from phycobiliproteins of dulse *Palmaria palmata*. *Marine Drugs*, *14*(2), 32. https://doi.org/10.3390/md14020032

George, G., & Mathew, L. (2017). Anti-oxidant potential of four Chlorophycean members from Kerala Coast. *International Journal of Scientific Research*, 6(7), 66-68.

Ghadiryanfar, M., Rosentrater, K. A., Keyhani, A., & Omid, M. (2018). Corrigendum to "A review of macroalgae production, with potential applications in biofuels and bioenergy" [Renew Sustain Energy Rev 54 (2016) 473–481]. *Renewable and Sustainable Energy Reviews*, *96*, 526. https://doi.org/10.1016/j.rser.2018.08.048

Gheda, S. F., El-Adawi, H. I., & El-Deeb, N. M. (2016). Antiviral profile of brown and red seaweed polysaccharides against hepatitis c virus. *Iranian Journal of Pharmaceutical Research*, *15*(3), 483–491. https://doi.org/10.22037/ijpr.2016.1944

Gnanavel, V., Roopan, S. M., & Rajeshkumar, S. (2019). Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. *Aquaculture*, *507*, 1–6. https://doi.org/10.1016/j.aquaculture.2019.04.004

Gómez-Guzmán, M., Rodríguez-Nogales, A., Algieri, F., & Gálvez, J. (2018). Potential role of seaweed polyphenols in cardiovascular-associated disorders. *Marine Drugs*, *16*(8), 250. https://doi.org/10.3390/md16080250

Gosch, B. J., Magnusson, M., Paul, N. A., & de Nys, R. (2012). Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. *GCB Bioenergy*, *4*(6), 919–930. https://doi.org/10.1111/j.1757-1707.2012.01175.x

Gullón, B., Gagaoua, M., Barba, F. J., Gullón, P., Zhang, W., & Lorenzo, J. M. (2020). Seaweeds as promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. *Trends in Food Science and Technology*, *100*, 1–18. https://doi.org/10.1016/j.tifs.2020.03.039

Gunathilaka, T. L., Samarakoon, K., Ranasinghe, P., & Peiris, L. D. C. (2020). Antidiabetic potential of marine brown algae - A mini review. *Journal of Diabetes Research*, *2020*, 1230218. https://doi.org/10.1155/2020/1230218

Gutiérrez-Rodríguez, A. G., Juárez-Portilla, C., Olivares-Bañuelos, T., & Zepeda, R. C. (2018). Anticancer activity of seaweeds. *Drug Discovery Today*, *23*(2), 434–447. https://doi.org/10.1016/j.drudis.2017.10.019

Ha, M., Morrow, M., & Algiers, K. (2021, June 8). Brown algae and diatoms. https://bio.libretexts.org/@go/page/31928

Haq, S. H., Al-Ruwaished, G., Al-Mutlaq, M. A., Naji, S. A., Al-Mogren, M., Al-Rashed, S., Ain, Q. T., Al-Amro, A. A., & Al-Mussallam, A. (2019). Antioxidant, anticancer activity and phytochemical analysis of green algae, chaetomorpha collected from the Arabian Gulf. *Scientific Reports*, *9*(1), 1–7. https://doi.org/10.1038/s41598-019-55309-1

Hardouin, K., Bedoux, G., Burlot, A. S., Donnay-Moreno, C., Bergé, J. P., Nyvall-Collén, P., & Bourgougnon, N. (2016). Enzyme-assisted extraction (EAE) for the production of antiviral and antioxidant extracts from the green seaweed *Ulva armoricana* (Ulvales, Ulvophyceae). *Algal Research*, *16*, 233–239. https://doi.org/10.1016/j.algal.2016.03.013

Hentati, F., Delattre, C., Ursu, A. V., Desbrières, J., Le Cerf, D., Gardarin, C., Abdelkafi, S., Michaud, P., & Pierre, G. (2018). Structural characterization and antioxidant activity of water-soluble polysaccharides from the Tunisian brown seaweed *Cystoseira compressa*. *Carbohydrate Polymers*, *198*, 589–600. https://doi.org/10.1016/j.carbpol.2018.06.098

Hentati, F., Tounsi, L., Djomdi, D., Pierre, G., Delattre, C., Ursu, A. V., Fendri, I., Abdelkafi, S., & Michaud, P. (2020). Bioactive polysaccharides from seaweeds. *Molecules*, *25*(14), 1–29. https://doi.org/10.3390/molecules25143152

Hermund, D. B. (2018). Antioxidant properties of seaweed-derived substances. In Qin, Y. (Ed.), *Bioactive Seaweeds for Food Applications: Natural Ingredients for Healthy Diets* (pp. 201-221). Elsevier Inc. https://doi.org/10.1016/B978-0-12-813312-5.00010-8

Jesumani, V., Du, H., Aslam, M., Pei, P., & Huang, N. (2019). Potential use of seaweed bioactive compounds in skincare—A review. *Marine Drugs*, *17*(12), 688. https://doi.org/10.3390/md17120688

Jiménez-Escrig, A., Gómez-Ordóñez, E., & Rupérez, P. (2011). Seaweed as a source of novel nutraceuticals: Sulfated polysaccharides and peptides. *Advances in Food and Nutrition Research*, *64*, 325–337. https://doi.org/10.1016/B978-0-12-387669-0.00026-0

Jumaidin, R., Sapuan, S.M., Jawaid, M., Shak, M.R., & Sahari, J. (2018). Seaweeds as Renewable Sources for Biopolymers and its Composites: A Review. *Current Analytical Chemistry*, *14*(3), 249-267. https://doi.org/10.2174/1573411013666171009164355

Kadam, S. U., Tiwari, B. K., Smyth, T. J., & O'Donnell, C. P. (2015). Optimization of ultrasound assisted extraction of bioactive components from brown seaweed Ascophyllum nodosum using response surface methodology. *Ultrasonics Sonochemistry*, *23*, 308–316. https://doi.org/10.1016/j.ultsonch.2014.10.007

Kalasariya, H. S., Yadav, V. K., Yadav, K. K., Tirth, V., Algahtani, A., Islam, S., Gupta, N., & Jeon, B. H. (2021). Seaweed-based molecules and their potential biological activities: An eco-sustainable cosmetics. *Molecules*, *26*(17), 5313. https://doi.org/10.3390/molecules26175313

Kendel, M., Wielgosz-Collin, G., Bertrand, S., Roussakis, C., Bourgougnon, N. B., & Bedoux, G. (2015). Lipid composition, fatty acids and sterols in the seaweeds ulva armoricana, and solieria chordalis from brittany (France): An analysis from nutritional, chemotaxonomic, and antiproliferative activity perspectives. *Marine Drugs*, *13*(9), 5606–5628. https://doi.org/10.3390/md13095606

Khalid, S., Abbas, M., Saeed, F., Bader-Ul-Ain, H., & Ansar Rasul Suleria, H. (2018). *Therapeutic Potential of Seaweed Bioactive Compounds. Seaweed Biomaterials*. IntechOpen. https://doi.org/10.5772/intechopen.74060

Kılınç, B., Cirik, S., Turan, G., Tekogul, H., & Koru, E. (2013). Seaweeds for food and industrial applications. In Muzzalupo, I. (Ed.), *Food Industry*. IntechOpen. https://doi.org/10.5772/53172

Kolanjinathan, K., Ganesh, P., & Saranraj, P. (2014). Pharmacological Importance of Seaweeds: A Review. *World Journal of Fish and Marine Sciences*, *6*(1), 1–15. https://doi.org/10.5829/idosi.wjfms.2014.06.01.76195

Kulshreshtha, G., Burlot, A. S., Marty, C., Critchley, A., Hafting, J., Bedoux, G., Bourgougnon, N., & Prithiviraj, B. (2015). Enzyme-assisted extraction of bioactive material from Chondrus crispus and codium fragile and its effect on Herpes simplex virus (HSV-1). *Marine Drugs*, *13*(1), 558–580. https://doi.org/10.3390/md13010558

Lee, W. K., Lim, Y. Y., Leow, A. T. C., Namasivayam, P., Ong Abdullah, J., & Ho, C. L. (2017). Biosynthesis of agar in red seaweeds: A review. *Carbohydrate Polymers*, *164*, 23–30. https://doi.org/10.1016/j.carbpol.2017.01.078

Liu, X., Wang, S., Cao, S., He, X., Qin, L., He, M., Yang, Y., Hao, J., & Mao, W. (2018). Structural characteristics and anticoagulant property in vitro and in vivo of a seaweed Sulfated Rhamnan. *Marine Drugs*, *16*(7), 243. https://doi.org/10.3390/md16070243

Lorenzo, J. M., Agregán, R., Munekata, P. E. S., Franco, D., Carballo, J., Şahin, S., Lacomba, R., & Barba, F. J. (2017). Proximate composition and nutritional value of three macroalgae: *Ascophyllum nodosum*, *Fucus vesiculosus* and *Bifurcaria bifurcata*. *Marine Drugs*, *15*(11), 360. https://doi.org/10.3390/md15110360

Lucas, W. J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S. R., Helariutta, Y., He, X. Q., Fukuda, H., Kang, J., Brady, S. M., Patrick, J. W., Sperry, J., Yoshida, A., López-Millán, A. F., Grusak, M. A., & Kachroo, P. (2013). The plant vascular system: Evolution, development and functions. *Journal of Integrative Plant Biology*, *55*(4), 294–388. https://doi.org/10.1111/jipb.12041

Luo, X., Su, P., & Zhang, W. (2015). Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications. *Marine Drugs*, *13*(7), 4231–4254. https://doi.org/10.3390/md13074231

Machmudah, S., Wahyudiono, Kanda, H., & Goto, M. (2018). Supercritical fluids extraction of valuable compounds from algae: Future perspectives and challenges. *Engineering Journal*, *22*(5), 13–30. https://doi.org/10.4186/ej.2018.22.5.13

Mahadevan, K. (2015). Seaweeds: A sustainable food source. In Tivari, K., & Troy, D. J. (Eds.), *Seaweed Sustainability: Food and Non-Food Applications* (pp. 347-364) Elsevier. https://doi.org/10.1016/B978-0-12-418697-2.00013-1

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, *212*, 1–17. https://doi.org/10.1016/j.anifeedsci.2015.09.018

Martínez–Hernández, G. B., Castillejo, N., Carrión–Monteagudo, M. del M., Artés, F., & Artés-Hernández, F. (2018). Nutritional and bioactive compounds of commercialized algae powders used as food supplements. *Food Science and Technology International*, *24*(2), 172–182. https://doi.org/10.1177/1082013217740000

Michalak, I., Górka, B., Wieczorek, P. P., Rój, E., Lipok, J., Łęska, B., Messyasz, B., Wilk, R., Schroeder, G., Dobrzyńska-Inger, A., & Chojnacka, K. (2016). Supercritical fluid extraction of algae enhances levels of biologically active compounds promoting plant growth. *European Journal of Phycology*, *51*(3), 243–252. https://doi.org/10.1080/09670262.2015.1134813

Michalak, I., Tuhy, Ł., & Chojnacka, K. (2015). Seaweed extract by microwave assisted extraction as plant growth biostimulant. *Open Chemistry*, *13*(1), 1183–1195. https://doi.org/10.1515/chem-2015-0132

Misra, N. N., Rai, D. K., & Hossain, M. (2015). Analytical techniques for bioactives from seaweed. In Tivari, K., & Troy, D. J. (Eds.), *Seaweed Sustainability: Food and Non-Food Applications* (pp. 271–287). Elsevier Inc. https://doi.org/10.1016/B978-0-12-418697-2.00010-6

Mišurcová, L., Ambrožová, J., & Samek, D. (2011). Seaweed lipids as nutraceuticals. *Advances in Food and Nutrition Research*, *64*, 339–355. https://doi.org/10.1016/B978-0-12-387669-0.00027-2

Montero, L., del Pilar Sánchez-Camargo, A., Ibáñez, E., & Gilbert-López, B. (2017). Phenolic compounds from edible algae: Bioactivity and health benefits. *Current Medicinal Chemistry*, *25*(37), 4808–4826. https://doi.org/10.2174/0929867324666170523120101

Morais, T., Cotas, J., Pacheco, D., & Pereira, L. (2021). Seaweeds compounds: An ecosustainable source of cosmetic ingredients? *Cosmetics*, *8*(1), 1–28. https://doi.org/10.3390/COSMETICS8010008

Morais, T., Inácio, A., Coutinho, T., Ministro, M., Cotas, J., Pereira, L., & Bahcevandziev, K. (2020). Seaweed potential in the animal feed: A review. *Journal of Marine Science and Engineering*, *8*(8), 1–24. https://doi.org/10.3390/JMSE8080559

Moubayed, N. M. S., Al Houri, H. J., Al Khulaifi, M. M., & Al Farraj, D. A. (2017). Antimicrobial, antioxidant properties and chemical composition of seaweeds collected from Saudi Arabia (Red Sea and Arabian Gulf). *Saudi Journal of Biological Sciences*, *24*(1), 162–169. https://doi.org/10.1016/j.sjbs.2016.05.018

Mouritsen, O. G., Rhatigan, P., & Pérez-Lloréns, J. L. (2018). World cuisine of seaweeds: Science meets gastronomy. *International Journal of Gastronomy and Food Science*, *14*, 55–65. https://doi.org/10.1016/j.ijgfs.2018.09.002

Murai, U., Yamagishi, K., Kishida, R., & Iso, H. (2021). Impact of seaweed intake on health. *European Journal of Clinical Nutrition*, *75*(6), 877–889. https://doi.org/10.1038/s41430-020-00739-8

Nakhate, P., & van der Meer, Y. (2021). A systematic review on seaweed functionality: A sustainable bio-based materia. *Sustainability*, *13*(11), 6174. https://doi.org/10.3390/su13116174

Naseri, A., Marinho, G. S., Holdt, S. L., Bartela, J. M., & Jacobsen, C. (2020). Enzyme-assisted extraction and characterization of protein from red seaweed Palmaria palmata. *Algal Research*, *47*, 101849. https://doi.org/10.1016/j.algal.2020.101849

Nasmia, Rosyida, E., Masyahoro, A., Putera F. H. A., Natsir, S. (2021). The utililzation of seaweed-based liquid organic fertilizer to stimulate *Gracilaria verrucosa* growth and quality. *International Journal of Environmental Science and Technology*, *18*, 1637-15644. https://doi.org/10.1007/s13762-020-02921-8

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). (2016). Hyperthyroidism (Overactive Thyroid). Retrieved on June 28, 2021, from https://www.nih.gov/

Nazarudin, M. F., Yusoff, F., Idrus, E. S., & Aliyu-Paiko, M. (2020). Brown seaweed Sargassum polycystum as dietary supplement exhibits prebiotic potentials in Asian sea bass *Lates calcarifer* fingerlings. *Aquaculture Reports*, *18*, 100488. https://doi.org/10.1016/j.aqrep.2020.100488

Nešić, A., Cabrera-Barjas, G., Dimitrijević-Branković, S., Davidović, S., Radovanović, N., & Delattre, C. (2019). Prospect of polysaccharide-based materials as advanced food packaging. *Molecules*, *25*(1), 135. https://doi.org/10.3390/molecules25010135

Olsson, J., Toth, G. B., & Albers, E. (2020). Biochemical composition of red, green and brown seaweeds on the Swedish west coast. *Journal of Applied Phycology*, *32*(5), 3305–3317. https://doi.org/10.1007/s10811-020-02145-w

Otero, P., Quintana, S. E., Reglero, G., Fornari, T., & García-Risco, M. R. (2018). Pressurized Liquid Extraction (PLE) as an innovative green technology for the effective enrichment of galician algae extracts with high quality fatty acids and antimicrobial and antioxidant properties. *Marine Drugs*, *16*(5), 156. https://doi.org/10.3390/md16050156

Pal, A., Kamthania, M. C., & Kumar, A. (2014). Bioactive compounds and properties of seaweeds—A Review. *Open Access Library Journal*, *01*(04), e752. https://doi.org/10.4236/oalib.1100752

Palstra, A. P., Kals, J., Garcia, A. B., Dirks, R. P., & Poelman, M. (2018). Immunomodulatory effects of dietary seaweeds in LPS challenged Atlantic Salmon *Salmo salar* as determined by deep RNA sequencing of the head kidney transcriptome. *Frontiers in Physiology*, *9*, 625. https://doi.org/10.3389/fphys.2018.00625

Peñalver, R., Lorenzo, J. M., Ros, G., Amarowicz, R., Pateiro, M., & Nieto, G. (2020). Seaweeds as a functional ingredient for a healthy diet. *Marine Drugs*, *18*(6), 301. https://doi.org/10.3390/md18060301

Pereira, L., & Cotas, J. (2019). Historical use of seaweed as an agricultural fertilizer in the European Atlantic area. In Pereira, L., Bahcevandziev, K., & Joshi, N. H. (Eds.), *Seaweeds as plant fertilizer, agricultural biostimulants and animal fodder* (pp. 1–22). CRC Press. https://doi.org/10.1201/9780429487156-1

Pérez, M. J., Falqué, E., & Domínguez, H. (2016). Antimicrobial action of compounds from marine seaweed. *Marine Drugs*, *14*(3), 1–38. https://doi.org/10.3390/md14030052

Pérez-López, P., Balboa, E. M., González-García, S., Domínguez, H., Feijoo, G., & Moreira, M. T. (2014). Comparative environmental assessment of valorization strategies of the invasive macroalgae *Sargassum muticum*. *Bioresource Technology*, *161*, 137–148. https://doi.org/10.1016/j.biortech.2014.03.013

Praveen, M. A., Parvathy, K. R. K., Balasubramanian, P., & Jayabalan, R. (2019). An overview of extraction and purification techniques of seaweed dietary fibers for immunomodulation on gut microbiota. *Trends in Food Science and Technology*, *92*, 46–64. https://doi.org/10.1016/j.tifs.2019.08.011

Premarathna, A. D., Amcp, K., Jayasooriya, A. P., De, J., & Adhikari, R. B. (2019a). Distribution and diversity of seaweed species in south coastal waters in Sri Lanka. *Journal of Oceanography and Marine Research*, *7*(S1), 196. https://doi.org/10.35248/2572-3103.19.7.196

Premarathna, A. D., Ranahewa, T. H., Wijesekera, S., & Wijesundera, K. K. (2019b). Wound healing properties of aqueous extracts of *Sargassum illicifolium* : An in vitro assay wound healing properties of aqueous extracts of *Sargassum illicifolium* : An in vitro assay. *Wound Medicine*, *24*(1), 1-7. https://doi.org/10.1016/j.wndm.2018.11.001

Quitain, A. T., Kai, T., Sasaki, M., & Goto, M. (2013). Microwave-hydrothermal extraction and degradation of fucoidan from supercritical carbon dioxide deoiled *Undaria pinnatifida*. *Industrial and Engineering Chemistry Research*, *52*(23), 7940–7946. https://doi.org/10.1021/ie400527b

Ramnani, P., Chitarrari, R., Tuohy, K., Grant, J., Hotchkiss, S., Philp, K., Campbell, R., Gill, C., & Rowland, I. (2012). Invitro fermentation and prebiotic potential of novel low molecular weight polysaccharides derived from agar and alginate seaweeds. *Anaerobe*, *18*(1), 1–6. https://doi.org/10.1016/j.anaerobe.2011.08.003

Rao, P. S., Periyasamy, C., Kumar, K. S., Rao, A. S., & Anantharaman, P. (2018). Seaweeds: Distribution, production and uses. In Noor, M. N., Bahtnagar, S. K., & Sinha, S. K. (Eds.), *Bioprospecting of algae-2018* (pp. 59-78). Society for Plant Research India.

Rengasamy, K. R., Mahomoodally, M. F., Aumeeruddy, M. Z., Zengin, G., Xiao, J., & Kim, D. H. (2020). Bioactive compounds in seaweeds: An overview of their biological properties and safety. *Food and Chemical Toxicology*, *135*, 111013. https://doi.org/10.1016/j.fct.2019.111013

Rhein-Knudsen, N., Ale, M. T., Ajalloueian, F., Yu, L., & Meyer, A. S. (2017). Rheological properties of agar and carrageenan from Ghanaian red seaweeds. *Food Hydrocolloids*, *63*, 50–58. https://doi.org/10.1016/j.foodhyd.2016.08.023

Rodrigues, D., Sousa, S., Silva, A., Amorim, M., Pereira, L., Rocha-Santos, T. A. P., Gomes, A. M. P., Duarte, A. C., & Freitas, A. C. (2015). Impact of enzyme- and ultrasound-assisted extraction methods on biological properties of red, brown, and green seaweeds from the Central West Coast of Portugal. *Journal of Agricultural and Food Chemistry*, *63*(12), 3177–3188. https://doi.org/10.1021/jf504220e

Rodriguez-Jasso, R. M., Mussatto, S. I., Pastrana, L., Aguilar, C. N., & Teixeira, J. A. (2011). Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed. *Carbohydrate Polymers*, *86*(3), 1137–1144. https://doi.org/10.1016/j.carbpol.2011.06.006

Ruban, P., & Govindasamy, C. (2018). Seaweed fertilizers in modern agriculture. *International Journal of Research Publications*, *14*(1), 100141102018386.

Rubio, C., Napoleone, G., Luis-González, G., Gutiérrez, A. J., González-Weller, D., Hardisson, A., & Revert, C. (2017). Metals in edible seaweed. *Chemosphere*, *173*, 572–579. https://doi.org/10.1016/j.chemosphere.2017.01.064

Ruggiero, M. A., Gordon, D. P., Orrell, T. M., Bailly, N., Bourgoin, T., Brusca, R. C., Cavalier-Smith, T., Guiry, M. D., & Kirk, P. M. (2015). A higher level classification of all living organisms. *PLoS ONE*, *10*(4), 1–60. https://doi.org/10.1371/journal.pone.0119248

Rupérez, P., Gómez-Ordóñez, E., & Jiménez-Escrig, A. (2013). Biological activity of algal sulfated and nonsulfated polysaccharides. In Hernández-Ledesma, B., & Herrero, M. (Eds.), *Bioactive Compounds from Marine Foods: Plant and Animal Sources* (pp. 219–247). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118412893.ch11

Sabeena, S. F., Alagarsamy, S., Sattari, Z., Al-Haddad, S., Fakhraldeen, S., Al-Ghunaim, A., & Al-Yamani, F. (2020). Enzyme-assisted extraction of bioactive compounds from brown seaweeds and characterization. *Journal of Applied Phycology*, *32*(1), 615–629. https://doi.org/10.1007/s10811-019-01906-6

Saito, H., & Lal, T. M. (2019). Antimycotic activity of seaweed extracts (*Caulerpa lentillifera* and *Eucheuma cottonii*) against two genera of marine oomycetes, *Lagenidium* spp. and *Haliphthoros* spp. *Biocontrol Science*, *24*(2), 73–80. https://doi.org/10.4265/BIO.24.73

Sánchez-Camargo, A. del P., Ibáñez, E., Cifuentes, A., & Herrero, M. (2017). Bioactives obtained from plants, seaweeds, microalgae and food by-products using pressurized liquid extraction and supercritical fluid extraction. *Comprehensive Analytical Chemistry*, *76*, 27–51. https://doi.org/10.1016/bs.coac.2017.01.001

Saraswati, Giriwono, P. E., Iskandriati, D., Tan, C. P., & Andarwulan, N. (2019). Sargassum seaweed as a source of anti-inflammatory substances and the potential insight of the tropical species: A review. *Marine Drugs*, *17*(10), 1–35. https://doi.org/10.3390/md17100590

Seca, A. M. L., & Pinto, D. C. G. A. (2018). Overview on the antihypertensive and anti-obesity effects of secondary metabolites from seaweeds. *Marine Drugs*, *16*(7), 237. https://doi.org/10.3390/md16070237

Seong, H., Bae, J. H., Seo, J. S., Kim, S. A., Kim, T. J., & Han, N. S. (2019). Comparative analysis of prebiotic effects of seaweed polysaccharides laminaran, porphyran, and ulvan using in vitro human fecal fermentation. *Journal of Functional Foods*, *57*, 408–416. https://doi.org/10.1016/j.jff.2019.04.014

Sevimli-Gur, C., & Yesil-Celiktas, O. (2019). Cytotoxicity screening of supercritical fluid extracted seaweeds and phenylpropanoids. *Molecular Biology Reports*, *46*(4), 3691–3699. https://doi.org/10.1007/s11033-019-04812-9

Shelar, P. S., Kumar, V., & Gauri, S. (2012). Medicinal value of seaweeds and its applications–A review. *Continental Journal of Pharmacology and Toxicology Research*, *5*(2), 1–22.

Silva, J., Alves, C., Pinteus, S., Mendes, S., & Pedrosa, R. (2018). Neuroprotective effects of seaweeds against 6-hydroxidopamine-induced cell death on an in vitro human neuroblastoma model. *BMC Complementary and Alternative Medicine*, *18*(1), 4–13. https://doi.org/10.1186/s12906-018-2103-2

Singh, S., Gaikwad, K. K., Park, S. Il, & Lee, Y. S. (2017). Microwave-assisted step reduced extraction of seaweed (*Gelidiella aceroso*) cellulose nanocrystals. International *Journal of Biological Macromolecules*, *99*, 506–510. https://doi.org/10.1016/j.ijbiomac.2017.03.004

Škrovánková, S. (2011). Seaweed vitamins as nutraceuticals. *Advances in Food and Nutrition Research*, *64*, 357–369. https://doi.org/10.1016/B978-0-12-387669-0.00028-4

Subaryono, S. (2018). Carageenan oligosaccharides: Biological activity and its development opportunities in Indonesia. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, *13*(1), 35-43.

Sudhakar, K., Mamat, R., Samykano, M., Azmi, W. H., Ishak, W. F. W., & Yusaf, T. (2018). An overview of marine macroalgae as bioresource. *Renewable and Sustainable Energy Reviews*, *91*, 165–179. https://doi.org/10.1016/j.rser.2018.03.100

Sun, Z., Dai, Z., Zhang, W., Fan, S., Liu, H., Liu, R., & Zhao, T. (2018). Antiobesity, antidiabetic, antioxidative, and antihyperlipidemic activities of bioactive seaweed substances. In Qin, Y. (Ed.), *Bioactive Seaweeds for Food Applications: Natural Ingredients for Healthy Diets* (pp. 239-253). Elsevier Inc. https://doi.org/10.1016/B978-0-12-813312-5.00012-1

Suryanarayana Murty, U., & Banerjee, A. K. (2011). Seaweeds: The wealth of oceans. In Kim, S. -K. (Ed.), *Handbook of Marine Macroalgae: Biotechnology and Applied Phycology* (pp. 36–44). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119977087.ch2

Susanto, E., Fahmi, A. S., Hosokawa, M., & Miyashita, K. (2019). Variation in lipid components from 15 species of tropical and temperate seaweeds. *Marine Drugs*, *17*(11), 630. https://doi.org/10.3390/md17110630

Teas, J., Vena, S., Cone, D. L., & Irhimeh, M. (2013). The consumption of seaweed as a protective factor in the etiology of breast cancer: Proof of principle. *Journal of Applied Phycology*, *25*(3), 771–779. https://doi.org/10.1007/s10811-012-9931-0

Thao My, P. Le, Sung, V. Van, Dat, T. Do, Nam, H. M., Phong, M. T., & Hieu, N. H. (2020). Ultrasound-assisted extraction of fucoidan from Vietnamese brown Seaweed *Sargassum mcclurei* and testing bioactivities of the extract. *ChemistrySelect*, *5*(14), 4371–4380. https://doi.org/10.1002/slct.201903818

van Wyk, A. S., & Prinsloo, G. (2020). Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. *South African Journal of Botany*, *133*, 54–62. https://doi.org/10.1016/j.sajb.2020.06.031

Vásquez, V., Martínez, R., & Bernal, C. (2019). Enzyme-assisted extraction of proteins from the seaweeds *Macrocystis pyrifera* and *Chondracanthus chamissoi*: characterization of the extracts and their bioactive potential. *Journal of Applied Phycology*, *31*(3), 1999–2010. https://doi.org/10.1007/s10811-018-1712-y

Vatsos, I. N., & Rebours, C. (2015). Seaweed extracts as antimicrobial agents in aquaculture. *Journal of Applied Phycology*, *27*(5), 2017–2035. https://doi.org/10.1007/s10811-014-0506-0

Venugopal, V. (2019). Sulfated and non-sulfated polysaccharides from seaweeds and their uses: An overview. *ECronicon Nutrition*, *2*, 126–141.

Woo, C. S. J., Lau, J. S. H., & El-Nezami, H. (2012). Herbal medicine. Toxicity and recent trends in assessing their potential toxic effects. In Shyur, L. F., & Lau, A. S. Y. (Eds.), *Advances in Botanical Research Volume 62* (pp. 365-384). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-394591-4.00009-X

Yeh, T. S., Hung, N. H., & Lin, T. C. (2014). Analysis of iodine content in seaweed by GC-ECD and estimation of iodine intake. *Journal of Food and Drug Analysis*, *22*(2), 189–196. https://doi.org/10.1016/j.jfda.2014.01.014

Yim, M. J., Lee, J. M., Choi, G., Cho, S. Y., & Lee, D. S. (2019). The antihyperlipidemic effect of alginate-free residue from sea tangle in hyperlipidemic rats. *Fisheries and Aquatic Sciences*, *22*(1), 4–9. https://doi.org/10.1186/s41240-019-0144-1

Yokoi, K., & Konomi, A. (2012). Toxicity of so-called edible hijiki seaweed (*Sargassum fusiforme*) containing inorganic arsenic. *Regulatory Toxicology and Pharmacology*, *63*(2), 291–297. https://doi.org/10.1016/j.yrtph.2012.04.006

Youssouf, L., Lallemand, L., Giraud, P., Soulé, F., Bhaw-Luximon, A., Meilhac, O., D'Hellencourt, C. L., Jhurry, D., & Couprie, J. (2017). Ultrasound-assisted extraction and structural characterization by NMR of alginates and carrageenans from seaweeds. *Carbohydrate Polymers*, *166*, 55–63. https://doi.org/10.1016/j.carbpol.2017.01.041

Yu, K. X., Jantan, I., Ahmad, R., & Wong, C. L. (2014). The major bioactive components of seaweeds and their mosquitocidal potential. *Parasitology Research*, *113*(9), 3121–3141. https://doi.org/10.1007/s00436-014-4068-5

Zamani, S., Khorasaninejad, S., & Kashefi, B. (2013). The importance role of seaweeds of some characters of plant. *International Journal of Agriculture and Crop Sciences*, *5*(16), 1789–1793.

Zava, T. T., & Zava, D. T. (2011). Assessment of Japanese iodine intake based on seaweed consumption in Japan: A literature-based analysis. *Thyroid Research*, *4*(1), 1–7. https://doi.org/10.1186/1756-6614-4-14

Zheng, J., Chen, Y., Yao, F., Chen, W., & Shi, G. (2012). Chemical composition and antioxidant/antimicrobial activities in supercritical carbon dioxide fluid extract of gloiopeltis tenax. *Marine Drugs*, *10*(12), 2634–2647. https://doi.org/10.3390/md1012263

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RESEARCH ARTICLE

Fine-tuning of protein extraction from wall-deficient *Chlamydomonas reinhardtii* using liquid nitrogen and sonication-assisted cell disruption

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ABSTRACT

Disruption methods used to extract proteins from the cell often require optimization in terms of yield increase and molecular integrity according to the cell type. Most cell lysis methods primarily target the cell wall. However, even for the wall-deficient strains, efficient extraction of molecules in or attached to membranous structures is a delicate process. In this study, we optimized the protein extraction technique for a cell wall deficient strain of *Chlamydomonas reinhardtii*, which is also a preferred material for most of the recombinant protein production studies. Liquid nitrogen (LN) was evaluated for efficient protein extraction from wall-less strain. The results were compared with sonic treatments, which were optimized in terms of applied power and duration. The results showed that sonication at 25% power for 20 seconds of three rounds provided optimum results for the protein integrity and extraction yield (74.13±2 µg/mL and 185.32±5 mg/g). Although LN has provided similar results in terms of protein content compared to sonication, (70.15±4.43 µg/mL and 175.37±11.09 mg/g maximum), it revealed low efficiency in extracting intact proteins from sub-compartments of the cell.

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Introduction

Algae have been used as a valuable food source for centuries due to their high protein, lipid, and pigment content (Kay, 1991; Wells et al., 2017; Torres-Tiji et al., 2020). Food scarcity caused by climate change in the world has increased the interest in algae as an alternative protein source to plant- and animal-based proteins (Chiong et al., 2016; Bleakley & Hayes, 2017). Microalgae species such as *Chlorella* sp. (Lai et al., 2019) and *Scenedesmus* sp. (Patnaik et al., 2019) are well known natural protein sources utilized as food supplements or feed additives. In addition, microalgae cells have been used effectively to produce recombinant proteins for many years (Gong et al.,

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2011; Rasala & Mayfield, 2015; Doron et al., 2016, Dyo & Purton, 2018). Proteins accumulated by the expression of exogenous genes can be localized in different parts of the cell. Some recombinant products can be stored inside the cytoplasm, while others can accumulate in membranous structures such as chloroplast or mitochondria. Therefore, it is important to consider both the complete lysis of all organelles and the protection of the target molecule for efficient cell disruption.

C. reinhardtii is a widely studied algal species as a model organism at both physiological and molecular levels (Hummel et al., 2012; Saloméa & Merchant, 2019). It is also a host for production of several recombinant proteins such as therapeutics, edible vaccines, or antimicrobials (Rosales-Mendosa et al., 2012; Ahmad et al., 2020). *C. reinhardtii* cell wall consists of hydroxyproline-rich glycoprotein layers (Goodenough & Heuser, 1985) that give robustness to this unicellular organism; however, it also constitutes an obstacle to deliver or extract material of interest.

Cell disruption techniques are widely studied for their cost-effectiveness, energy consumption, and molecular integrity of the target product (D'Hondt et al., 2017; Dixon & Wilken, 2018; Sotto-Sierra et al., 2018). Enzymatic treatment is one of the simple methods used to eliminate the cell wall. *C. reinhardtii* produces its own enzyme called autolysin during the vegetative and sexual stages of its life cycle to lyse the wall structure occurs around zoospores, zygospores and gametes (Jaenicke et al., 1987). However, the use of this enzyme for disruption can result in partial lysis of the cell and may require long incubation periods. In addition, the enzyme can either be bought commercially, which increases the implementation costs, or it can be produced from microalgae itself, which adds an extra step to the disruption process. Cell wall-less species of microalgae such as *Dunaliella salina* is easier to study in terms of cell disruption. Similarly, using a mutant strain without a cell wall is also a preferable strategy providing an advantage for breaking the cell (Lam et al., 2017).

Freeze-thaw is a simple disruption technique for creating pores in the cell wall and membranes during the thawing of the ice crystals that occurred at the freezing step (Dixon & Wilken, 2018). Liquid nitrogen (LN) provides fast freeze of the cells due to extremely low boiling point (-196°C). Sonication, on the other hand, is based on the formation of cavitation bubbles by ultrasound waves and disruption of the cells by mechanical shear stress (Avhad et al., 2014). Sonication stands out as a more powerful technique with moderate energy consumption for breaking the cell wall and internal membranes compared to the freeze-thaw method, however, it is usually preferred to use two techniques together or to use them in combination with other techniques to obtain better results (Gerde et al., 2012; Sotto-Sierra et al., 2018; Stirk et al., 2020). Both methods have the advantage of avoiding the use of chemicals or the need to remove external materials such as beads. It is also possible to apply heat control in sonication to protect degradable molecules such as proteins and lipids. In this study, the LN and sonication methods were compared and optimized for protein isolation from a wall deficient strain of *C. reinhardtii* concerning yield and structural integrity

Materials and Methods

Strain, Media, and Culture Conditions

C. reinhardtii strain cc-3395 cwd arg7-8 mt- (cell wall deficient strain carrying mutation on ARG7 gene encoding argininosuccinate lyase) was ordered from Chlamydomonas Resource Center (chlamycollection.org). The cells were first grown on agar plates containing TAP medium (Harris, 1989) with addition of 100 µg/mL arginine. A loop of cells was scraped from the plate and inoculated in a 100 mL SGII medium (Sager & Granick, 1953) containing 100 µg/mL arginine (SGII-A) in 250 mL flask. This culture was grown for 6 days on a shaker at 100 rpm under 30 µmol photon m−2 s−1 illumination at 25°C and utilized as the stock culture for the inoculation of larger-scale production. The stock culture was washed twice with fresh SGII-A medium and re-suspended in 30 mL of fresh medium before inoculation of 1.55 L final SGII-A culture medium in a 2 L glass bottle. Mixing and aeration was supplied to the culture by an air pump through a 2 µm filter. The culture was grown for 6 days under the same conditions provided for stock culture. The culture was aliquoted into 50 mL falcon tubes and used in disruption experiments (Figure 1).

Cell Density Measurements

Cell counts were performed at the beginning and the end of the cultivation by a Neauber hemocytometer. Dry weight per mL of the culture was identified by filtration of 50 mL of grown culture and drying the biomass at 65°C overnight. The culture was started with 3.2×10^5 cell/mL and ended at 1.19×10^6 cell/mL on the final day.

Sonication-Assisted Disruption

Three replicates of four falcon tubes with 50 mL of the culture (12 falcons in total) were centrifuged at 4000 rpm at

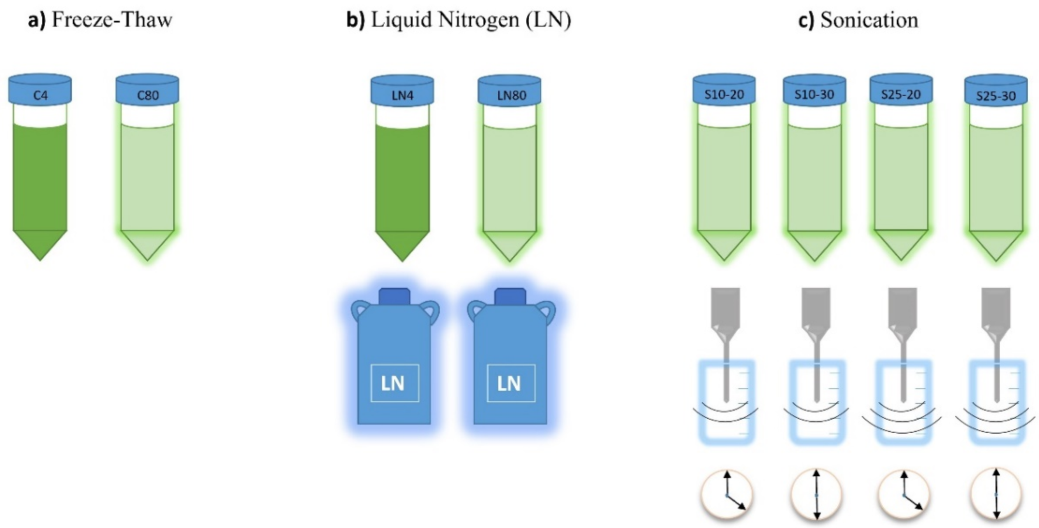

Figure 1. Graphical abstract of the experimental setup

+4°C and cell pellet was kept at -80°C overnight. Frozen cells were partially dissolved on ice and 500 µL of cold TBS containing 0.05% Tween 20 and 1 mM phenylmthylsulfonyl fluoride (PMSF) was added to resuspend and dissolve all the pellet. These cell-solution mixes were transferred into 1.5 mL Eppendorf tubes and kept on ice during the sonication process. Cell disruption was performed by using a 3 mm sonicator probe tip (Bandelin Sonoplus 2070: 70W HF power, 20 kHz) with a 5 cycle, 10% or 25% power, 20 sec or 30 sec durations for 3 complementary rounds (3×) with 10 seconds of resting time in between the rounds for each tube (Table 1). 50 µL of the lysed cells were separated for the later use at microscopic observations. The remaining lysates were centrifuged at 13,400 rpm (max) at +4°C for 25 minutes. Supernatants containing total soluble proteins (TSP) were transferred to new 1.5 mL Eppendorf tubes and kept at +4°C until protein analysis.

Conventional Freeze-Thawing and Use of Liquid Nitrogen

Three replicates of four falcon tubes containing 50 mL of the culture (12 falcons in total) were centrifuged at 4000 rpm at +4°C. Six of the cell pellets were kept at +4°C and remaining 6 of them were frozen at -80°C overnight. All samples were placed on ice and 500 µL of cold TBS containing 0.05% Tween 20 and 1 mM PMSF was added after partial thawing of the -80°C samples. Thawing was completed by continuous pipetting on ice and samples transferred into 1.5 mL Eppendorf tubes. Three replicates from each +4°C and -80°C samples were frozen the second round in liquid nitrogen (LN) for 10 seconds and were partially thawed at room temperature for 6 minutes then placed on ice (Table 1). After completing the thawing process, 50 µL of the lysed cells were separated for microscopic analysis. Remaining lysates were prepared for protein analysis as described for sonication.

Fluorescent microscopy

20 µL of each of the lysed sample was prepared on glass slide for microscopic observations. Chlorophyll autofluorescence was captured at 450 nm excitation spectrum using Leica DM4000B LED fluorescent microscope (Leica, Wetszlar, Germany) and Leica imaging software.

Protein quantification and PAGE analysis

Protein quantifications were measured by Bradford assay (Bradford, 1976). Two standard curves were generated to get more accurate estimations using Bovin Serum Albumin (BSA) as the standard protein molecule; the first one with 10, 20, 30, 40 and 50 µg/mL BSA for smaller amount of protein quantifications as in the control sample (C4) and the second curve with 100, 200, 300, 400 and 500 µg/mL BSA for larger quantities (rest of the samples). 2× Laemmli Buffer with 10% mercaptoethanol was added on to 10 µl of each extract and denatured at 95°C for 5 minutes before gel loading. All 20 µL of each sample was loaded to the gel. PageRuler™ Prestained Protein Ladder 10 to 180 kDa (ThermoFisher Scientific #26616) was loaded to the first lane as protein marker. Proteins were separated on 4-20% polyacrylamide gel (Miniprotean® TGXTM Precast Gels, BioRad, U.S.) at 120 volts and stained in Coomassie Brilliant Blue for 15 minutes and destained overnight on a shaker at 120 rpm.

Table 1. Description of freeze-thaw, liquid nitrogen and sonication methods applied in this study

Freeze-Thaw Control	Parameters
C4	+4°C overnight
C80	-80°C overnight
Liquid Nitrogen	
LN4	C4 + Liquid Nitrogen (-196°C)
LN80	C80 + Liquid Nitrogen (-196°C)
Sonication	
S10-20	C80 + Sonication; 10% power, 20 seconds, 3 rounds
S10-30	C80 + Sonication; 10% power, 30 seconds, 3 rounds
S25-20	C80 + Sonication; 25% power, 20 seconds, 3 rounds
S25-30	C80 + Sonication; 25% power, 30 seconds, 3 rounds

Table 2. Mean values of protein concentrations obtained from each disruption experiment

	µg/mL protein in liquid culture	mg/g protein per dry weight
Freeze-Thaw Control		
C4	0.98+0.24	2.46±0.60
C80	49.11±3.65	122.78±9.12
Liquid Nitrogen		
LN4	62.07±1.72**	155.18±4.29**
LN80	70.15±4.43*	175.37±11.09*
Sonication		
S10-20	59.57±2.95	148.93±7.39
S10-30	60.14±2.42	150.35±6.05
S25-20	74.13±2.00**	185.32±5.00**
S25-30	73.79±5.44**	184.46±13.60**

Note: * indicates significance level is 0.05; ** indicates significance level is 0.01

Statistical analysis

Experiments were conducted as three independent replicates. Statistical significance ($p<0.05$) between groups were determined by two-tailed t-test analysis using Microsoft Excel.

Results and Discussion

Protein concentrations

The results obtained from protein quantification analysis are summarized in Table 2. A considerably higher protein yield was obtained from the cells pre-frozen overnight at -80 degrees (C80) compared to the control culture at +4°C (C4). Liquid nitrogen (LN4) provided significantly higher protein yield compared to both results obtained from C4 and C80 samples ($p<0.005$ and $p<0.05$, respectively) (Figure 2). Remarkably, the results obtained by LN revealed similar amount of protein to the trials performed by sonication.

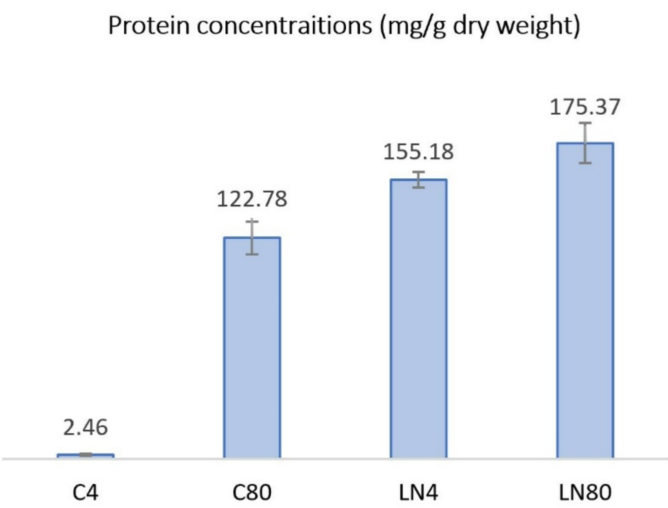

Figure 2. Protein concentrations from the freeze-thaw and LN experiments. (See Table 1 for the codes)

No significant difference for protein concentrations was observed between LN4 and 10% power (S10-20, S10-30) or LN80 and 25% power sonication treatments (S25-20, S25-30), regardless of the duration. Sonication treatments were evaluated in terms of both the level of power and duration of the application (Figure 3). The applied power rather than the application time emerged as a more important factor for protein recovery. Both 25% power applied samples with 20 and 30 seconds of application times (S25-20, S25-30) provided a higher amount of protein than the samples subjected to disruption with 10% power at the same durations (S10-20, S10-30) ($p<0.05$). Comparing the results of all disruption experiments, sonication for 20 seconds of three rounds using 25% power (S25-20) provided the highest amount of protein in this study (74.13±2 µg/mL and 185.32±5 mg/g).

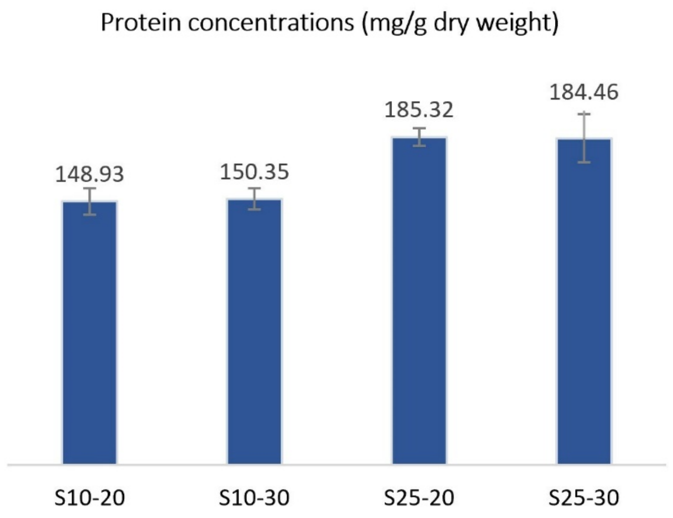

Figure 3. Protein concentrations from the samples sonicated in different application conditions (See Table 1 for the codes)

SDS PAGE analysis

10 µl of each protein extract was loaded on the gel for polyacrylamide gel electrophoresis (PAGE) analysis. The results showed consistency in terms of the concentration of extracted proteins. However, differences were observed in the protein bands obtained from different disruption techniques. The protein bands above 180 kDa (Figure 4, frame 1: *f1*) and around 25 kDa (Figure 4, frame 2: *f2*), provided different results between the application of LN and sonication. A partial degradation was also observed in between the sonication trials (S25-30) for the protein bands in *f1* depending on the duration.

Disruption efficiency

The autofluorescence property of the chlorophylls inside the cell was used to observe the cell lysis efficiency (Figure 5). While the integrity of the cells was preserved in the C4 control, the cells in the C80 samples were visualized as dispersed clusters. Chlorophyll fluorescence could still be observed in the samples that are disrupted with liquid nitrogen. Chlorophyll release was very low in only freeze-thawed samples (data not shown), and autofluorescence was preserved in intracellular structures. On the other hand, very weak autofluorescence signal was obtained from the sonicated samples, which were observed as pale cell residues.

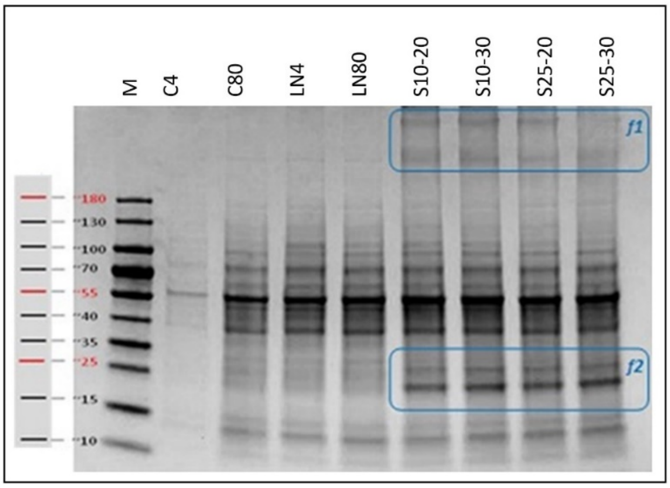

Figure 4. Protein profiles of the disrupted samples obtained from SDS PAGE analysis

Discussion

Proteins constitute approximately 40-60% of microalgae cells (Wang & Yin, 2018). Several techniques have been evaluated as a protein extraction method for *C. reinhardtii* (Newman et al., 1991; Bensalem et al., 2020). Cell wall removal is the main objective in most the cell disruption studies. Extraction of proteins from within the cell begins with cell lysis. Species with a cell wall require more rigorous methods, and the lack of this structure provides an advantage in terms of extraction success. In a previous study, Lam et al. (2017) obtained 3 times higher results with the cell wall-deficient strain of *C. reinhardtii* than the wild type in their study, in which they evaluated the effectiveness of the pulsed electric field (PEF) method in cell disruption. Removing the cell wall with autolysin pre-treatment was also reported to result a significantly higher yield of proteins Sotto-Sierra et al. (2017). Nevertheless, the complete disintegration of the membranous structures in the cell and the separation of proteins without degradation is a sensitive process. In this study, the effects of easy to apply freeze-thaw method, liquid nitrogen use and sonication application, in terms of protein extraction and molecular integrity for a wall-deficient strain of *C. reinhardtii* were compared.

Freeze-thawing technique is usually used in combination with most of the disruption methods. Freezing cells in very low

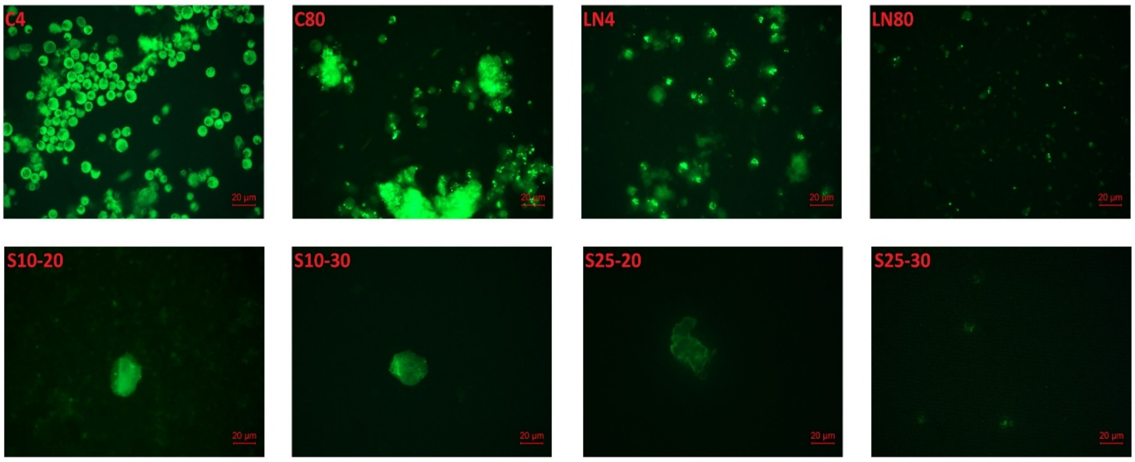

Figure 5. Chlorophyll autofluorescence of each sample after the disruption treatment

temperatures followed by thawing may damage the cell wall in the media due to melting of the ice crystals. However, its effect on the cell membranes is limited. The first step of this study was to investigate the concentration of proteins that can be extracted by keeping the wall-less cells at -80°C overnight (C80).

Disrupting the cells with liquid nitrogen after -80°C freezing step (LN80) resulted the highest amount of protein yield in between the freeze-thawing trials (Figure 2). Quantification analysis using liquid nitrogen revealed similarities with the sonication trials, indicating that freeze-thawing cells with LN would be sufficient to provide proteins for the wall-deficient strain of *C. reinhardtii*.

Sonication is one of the most effective mechanical disruption techniques that can effectively eliminate microalgal cell walls, which may contain cellulose, polysaccharides, and glycoproteins (Kuhavichanan et al., 2018; Alhattab et al., 2019). However, in this study, it was revealed that even in the absence of a cell wall, cell lysis should be planned with care for effective product recovery. Ultrasonic power and processing time are two important parameters for extracting high-value products from microorganisms, including microalgae. (Zheng et al., 2021). Kuhavichanan et al. (2018) reported that increasing the processing time of sonication treatment resulted in higher protein yields for green microalga *Coelastrum* sp. However, in our study, no significant difference was found between the times applied as 20 seconds for 3 rounds (60s in total) and 30 seconds for 3 rounds (90s in total). Instead, higher protein yield was achieved by the increase of applied power (percent amplitude).

Extraction efficiency and molecular integrity was also observed by the SDS PAGE analysis. The weak protein bands observed in C4 were presumably due to partial lysis occurred during centrifugation. The freeze-thaw method alone was insufficient for the extraction of protein bands above 180 kDa (Figure 4, frame 1: *f1*) and around 25 kDa (Figure 4, frame 2: *f2*), even using liquid nitrogen. Protein degradation occurred as the power increased in sonication applications with longer duration (S25-30) for the protein bands in *f1*. 55 kDa and 15 kDa bands of RubisCo protein (Sudhani et al., 2015) were observed in all samples Figure 4. RubisCo is an important protein responsible for carbon fixation in photosynthetic organisms and is localized in the chloroplast stroma in *C. reinhardtii* or in membrane-free mini-organelles called pyrenoids, which are also located within the chloroplast (Borkhsenious et al., 1998). The faint protein bands of about 25 kDa (*f2*) in freeze-thawed samples was presumably due to protein degradation. It is also possible that these bands are coming from subunits or organelles such as mitochondria, which have a small and double-layered membrane structure and therefore are more difficult to lyse with the freeze-thaw method. On the other hand, the protein bands at the same size have been previously reported to be the light-harvesting complex (LHCII) by Sotto-Sierra (2017) referring to White & Melis (2006). LHC proteins are known to be localized in thylakoid membranes of chloroplast in microalgae (Grewe et al., 2014). As a result, sonication turned out to be more effective than freeze-thaw methods, including liquid nitrogen, for complete lysis of the chloroplast.

Chlorophyll autofluorescence is a technique being used to easily detect the photosynthetic efficiency simply by measuring the excess light re-emitted by the chlorophyll molecules during photosynthesis (Maxwell & Johnson 2000). In our study, the autofluorescence of the chlorophylls was used to observe the cell lysis efficiency (Figure 5). Both LN and sonication assisted disruption methods were able to disperse the cell. However, the weak chlorophyll signals in LN treated samples were presumed to be pigments stuck into the partially disrupted chloroplast. To ensure the efficiency of LN treatment for the wall-deficient strain, further examination in a subcellular level should be conducted.

Conclusion

Microalgae are one of the important sources of natural proteins. Besides their nutritional value, they are also used successfully as a host system for recombinant protein production. It is important to determine the effective method and conditions for the extraction of proteins produced in microalgae. Although wall-deficient strains stand as easier to disintegrate, more elaborate and optimized methods need to be developed for the isolation of sensitive molecules such as proteins. In overall evaluation of this research, considering both the protein concentration and the molecular composition, the most efficient method to extract proteins from the cell wall-deficient *C. reinhardtii* was found to be by the sonication treatment at 25% power for 20 seconds for 3 rounds. Liquid nitrogen use as the second round of freeze-thawing was promising for obtaining high protein yield for this strain. However, using two techniques in combination (freeze-thaw and sonication) resulted in better end products. Since the strain choice of this study is also a well-known and highly preferred material for recombinant protein expression studies, these findings may serve the scientific community who works in that research field as well.

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Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Ahmad, N., Mehmood, M. A., & Malik S. (2020). Recombinant protein production in microalgae: emerging trends. *Protein & Peptide Letters*, *27*(2), 105–110. http://doi.org/10.2174/0929866526666191014124855

Alhattab, M., Kermanshahi-Pour, A., & Brooks, M. S. -L. (2019) Microalgae disruption techniques for product recovery: Influence of cell wall composition. *Journal of Applied Phycology*, *31*, 61–88. https://doi.org/10.1007/s10811-018-1560-9

Avhad, D. N., Niphadkar, S. S., & Rathod, V. K. (2014). Ultrasound assisted three phase partitioning of a fibrinolytic enzyme. *Ultrasonics Sonochemistry*, *21*(2), 628–633. http://doi.org/10.1016/j.ultsonch.2013.10.002

Bensalem, S., Pareau, D., Cinquin, B., Francaiz, O., Le Pioufle, B., & Lopes, F. (2020). Impact of pulsed electric fields and mechanical compressions on the permeability and structure of *Chlamydomonas reinhardtii* cells. *Scientific Reports*, *10*, 2668. https://doi.org/10.1038/s41598-020-59404-6

Bleakley, S., & Hayes, M. (2017). Algal proteins: Extraction, application and challenges concerning production. *Foods*, *6*(5), 33. https://doi.org/10.3390/foods6050033

Borkhsenious, O. N., Mason, C. B., & Moroney, J. V. (1998). The intracellular localization of ribulose-1,5-bisphosphate carboxylase/oxygenase in *Chlamydomonas reinhardtii*. *Plant Physiology*, *116*(4), 1585–1591. https://doi.org/10.1104/pp.116.4.1585

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Chiong, T., Acquah, C., Lau, S. Y., Khor, E. H., & Danquah, M. K. (2016). Microalgal-based protein by-products: Extraction, purification, and applications. In Dhillon, G. S. (Ed.), *Protein Byproducts; Transformation from Environmental Burden into Value-Added Products* (pp. 213-234). Academic Press. http://doi.org/10.1016/B978-0-12-802391-4.00012-4

D'Hondt, E., Martin-Juarez, J., Bolado, S., Kasperoviciene, J., Koreiviene, J., Sulcius, S., Elst, K., Bastiaens, L. (2017), Cell disruption technologies. In Gonzalez-Fernandez, C., & Muñoz, R. (Eds.), *Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End Products* (pp. 133-154). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-101023-5.00006-6

Dixon, C., & Wilken, L. R. (2018). Green microalgae biomolecule separations and recovery. *Bioresources and*

Bioprocessing, *5*, 14. https://doi.org/10.1186/s40643-018-0199-3

Doron, L., Segal, N., & Shapira, M. (2016). Transgene expression in microalgae-from tools to applications. *Frontiers in Plant Science*, *7*, 505. https://doi.org/10.3389/fpls.2016.00505

Dyo, Y. M., & Purton S. (2018). The algal chloroplast as a synthetic biology platform for production of therapeutic proteins. *Microbiology*, *164*(2), 113–121. https://doi.org/10.1099/mic.0.000599

Gerde, J. A., Montalbo-Lomboy, M., Yao L., Grewell, D., & Wang, T. (2012). Evaluation of microalgae cell disruption by ultrasonic treatment. *Bioresource Technology*, *125*, 175–181. https://doi.org/10.1016/j.biortech.2012.08.110

Gong, Y., Hu, H., Gao, Y., Xu, X., & Gao, H. (2011). Microalgae as platforms for production of recombinant proteins and valuable compounds: progress and prospects. *Journal of Industrial Microbiology and Biotechnology*, *38*(12), 1879–1890. https://doi.org/10.1007/s10295-011-1032-6

Goodenough, U. W., & Heuser, J. E. (1985). The Chlamydomonas cell wall and its constituent glycoproteins analyzed by the quick-freeze, deep-etch technique. *Journal of Cell Biology, 101*(4), 1550–1568. https://doi.org/10.1083/jcb.101.4.1550

Grewe, S., Ballottari, M., Alcocer, M., D'Andrea, C., Blifernez-Klassen, O., Hankamer, B., Mussgnug, J. H., Bassi, R., & Krusea, O. (2014). Light-harvesting complex protein LHCBM9 is critical for photosystem II activity and hydrogen production in *Chlamydomonas reinhardtii*. *The Plant Cell*, *26*(4), 1598–1611. https://doi.org/10.1105/tpc.114.124198

Harris, E. H. (1989). *The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use*. Academic Press. 780p. https://doi.org/10.1126/science.246.4936.1503-a

Hummel, E., Guttmann, P., Werner, S., Tarek, B., Schneider, G., Kunz, M., Frangakis, A. S., & Westermann, B. (2012). 3D ultrastructural organization of whole *Chlamydomonas reinhardtii* cells studied by nanoscale soft X-ray tomography. *Plos One*, *7*(12), e53293. https://doi.org/10.1371/journal.pone.0053293

Jaenicke, L., Kuhne, W., Spessert, R., Wahle, U., & Waffenschmidt, S. (1987). Cell-wall lytic enzymes (autolysins) of *Chlamydomonas reinhardtii* are (hydroxy)proline-specific proteases. *European Journal of Biochemistry*, *170*(1-2), 485-491. https://doi.org/10.1111/j.1432-1033.1987.tb13725.x

Kay, R. A. (1991). Microalgae as food and supplement. *Critical Reviews in Food Science and Nutrition*, *30*(6), 555-573. http://doi.org/10.1080/10408399109527556

Kuhavichanan, A., Kusolkumbot, P., Sirisattha, S., & Areeprasert, C. (2018). Mechanical extraction of protein solution from microalgae by ultrasonication. *IOP Conference Series: Earth and Environmental Science*, *159*, 012009. https://doi.org/10.1088/1755-1315/159/1/012009

Lai, Y. C., Chang, C. H., Chen, C. Y., Chang, J. S., & Ng, I. S. (2019). Towards protein production and application by using *Chlorella* species as circular economy. *Bioresource Technology*, *289*, 121625. https://doi.org/10.1016/j.biortech.2019.121625

Lam, P. G., Kolk, J. A., Chordia, A., Vermue, H. M., Olivieri, G., Eppink, M. H. M., & Wijffels, R. H. (2017). Mild and selective protein release of cell wall deficient microalgae with pulsed electric field. *ACS Sustainable Chemistry Engineering*, *5*(7), 6046-6053. https://doi.org/10.1021/acssuschemeng.7b00892

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany*, *51*(345), 659-668. https://doi.org/10.1093/jexbot/51.345.659

Newman, S. M., Gillham, N. W., Harris, E. H., Johnson, A. M., & Boynton, J. E. (1991). Targeted disruption of chloroplast genes in *Chlamydomonas reinhardtii*. *Molecular and General Genetics MGG*, *230*, 65–74. https://doi.org/10.1007/BF00290652

Patnaik, R., Singh, N. K., Bagchi, S. K., Rao, P. S., & Mallick, N. (2019). Utilization of Scenedesmus obliquus protein as a replacement of the commercially available fish meal under an algal refinery approach. *Frontiers in Microbiology*, *10*, 2114. https://doi.org/10.3389/fmicb.2019.02114

Rasala, B. A., & Mayfield, S. P. (2015). Photosynthetic biomanufacturing in green algae; production of recombinant proteins for industrial, nutritional, and medical uses. *Photosynthesis Research*, *123*(3), 227-39.https://doi.org/10.1007/s11120-014-9994-7

Rosales-Mendoza, S., Paz-Maldonado, L. M. T., & Soria-Guerra, R. E. (2012). *Chlamydomonas reinhardtii* as a viable platform for the production of recombinant proteins: Current status and perspectives. *Plant Cell Reports, 31*, 479-494 http://doi.org/10.1007/s00299-011-1186-8

Sager, R., & Granick, S. (1953). Nutritional studies with *Chlamydomonas reinhardtii*. *Annals of the New York Academy of Sciences*, *56*(5), 831-838. https://doi.org/10.1111/j.1749-6632.1953.tb30261.x

Saloméa, P. A., & Merchant, S. S. (2019). A series of fortunate events: Introducing *Chlamydomonas* as a reference organism. *Plant Cell, 31*, 1682–1707. https://doi.org/10.1105/tpc.18.00952

Sotto-Sierra, L., Dixon, C. K., & Wilken, L. R. (2017). Enzymatic cell disruption of the microalgae *Chlamydomonas reinhardtii* for lipid and protein extraction. *Algal Research*, *25*, 149–159. https://doi.org/10.1016/j.algal.2017.04.004

Sotto-Sierra, L., Stoykovab, P., & Nikolova, Z. L. (2018). Extraction and fractionation of microalgae-based protein products. *Algal Research, 36*, 175–192. https://doi.org/10.1016/j.algal.2018.10.023

Stirk, W. A., Bálint, P., Vambe, M., Lovász, C., Molnár, Z., van Staden, J., & Ördög, V. (2020). Effect of cell disruption methods on the extraction of bioactive metabolites from microalgal biomass. *Journal of Biotechnology, 307*, 35-43. https://doi.org/10.1016/j.jbiotec.2019.10.012

Sudhani, H. P., García-Murria, M. J., Marín-Navarro, J., García-Ferris, C., Peñarrubia, L., & Moreno, J. (2015). Purification of Rubisco from *Chlamydomonas reinhardtii. Bio-Protocol,* *5*(23), e1673. https://doi.org/10.21769/BioProtoc.1673

Torres-Tiji, Y., Fields, F. J., & Mayfield, S. P. (2020). Microalgae as a future food source. *Biotechnology Advances, 41*, 107536. https://doi.org/10.1016/j.biotechadv.2020.107536

Wang, J., & Yin, Y., (2018). Fermentative hydrogen production using pretreated microalgal biomass as feedstock. *Microbial Cell Factories, 17*, 22. https://doi.org/10.1186/s12934-018-0871-5

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E., & Brawley, S. H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology, 29*(2), 949–982. https://doi.org/10.1007/s10811-016-0974-5

White, A. L., & Melis, A. (2006). Biochemistry of hydrogen metabolism in *Chlamydomonas reinhardtii* wild type and a Rubisco-less mutant. *International Journal of Hydrogen Energy,* 31(4), 455–464. https://doi.org/10.1016/j.ijhydene.2005.04.028

Zheng, S., Zhang, G., Wang, H., Long, Z., Wei, T., & Li, Q. (2021). Progress in ultrasound-assisted extraction of the value-added products from microorganisms. *World Journal of Microbiology and Biotechnology*, *37*, 71. https://doi.org/10.1007/s11274-021-03037-y

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RESEARCH ARTICLE

On the hydrodynamic effects of the eidonomy of the hammerhead shark's cephalofoil in the eye bulb region: Winglet-like behaviour

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ABSTRACT

External morphology (eidonomy) of marine creatures, developed by the evolution process over the course of millions of years, plays a crucial role in their locomotion and swimming performance. In this paper, hydrodynamic impacts of the cephalofoil tip eidonomy (tip bump) in the eye bulb region of a scalloped hammerhead shark, *Sphyrna lewini*, are studied with the aid of computational fluid dynamics (CFD). In this regard, two separate geometries are designed here; one corresponding to the real geometry of the hammerhead shark's cephalofoil with a tip bump (eye bulb region) and another one, a modified version with a flat tip without the aforementioned bump. Turbulent flows encountered in the problem are simulated using the Lam-Bremhorst turbulence model at different angles of attack (AoA) and a sideslip angle, at high Reynolds number, 10^6, corresponding to the swimming of a juvenile hammerhead shark with a speed of 1 m/s. The results show that the strength (circulation) of the wing tip vortices reduces by the external geometry of the hammerhead's cephalofoil tip; in this sense, 'cephalofoil tip' with its unique morphology behaves as a winglet.

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Introduction

In general, two major factors determine the swimming performance and locomotion of swimming animals, including: animal's body deflection as a function of time (i.e., deflection dynamics) and animal's external morphology or eidonomy (Taheri, 2021a). Many marine faunas such as fishes (including sharks and rays), cetaceans (whales and dolphins), and jellyfish, utilize the concept of repetitive 'body deflection' in vast varieties to propel themselves in the aquatic environment. Computational fluid dynamics (CFD) can be successfully adopted to simulate fluid flow generated in the downstream wake of a swimming animal and also locomotion and propulsion generation by aquatic animals; such as anguilliform and carangiform swimmers (Borazjani, 2008), jellyfish (Taheri, 2018a; Miles and Battista, 2019), swimming nematode (Battista,

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2020; Taheri, 2021b) and manta rays (Fish et al., 2016), to name a few.

External morphology (eidonomy) of a swimming animal in interaction with its habitat (aquatic environment), as a major factor, determines important fluid flow phenomena encountered in the swimming process, such as: vortical structure generation and merger, flow separation and reattachment, etc. In other words, the presence of some morphological features on the external surface of the animal's body can potentially bring some favourable hydrodynamic effects for locomotion and swimming of the animal. For example, a wavy/undulatory geometric pattern at the leading edge of humpback whale's flippers leads to the formation of streamwise vortices; this leads to a stall delay and can typically provide better hydrodynamic performance at post-stall region (Miklosovic, 2004; Taheri, 2018b). As other instances of hydrodynamic effects of the external morphology, one can consider effects of ventral pleats on the belly of humpback whales (Taheri, 2018c), denticles on the sharkskin (Domel et al., 2018), ridges on the carapace of leatherback turtles (Bang et al., 2016) and longitudinal ridges on the dorsal area of whale sharks (Taheri, 2020). As an example of adverse (unfavourable) hydrodynamic effects of the external morphology on the locomotion, one can look at the effects of manta ray injuries (geometric deficiencies), generated by predator's attacks or boat strikes, on the manta ray gliding performance (Taheri, 2021c). In the present paper, external geometry of the scalloped hammerhead shark's cephalofoil is examined for possible favourable hydrodynamic characteristics.

Hammerhead sharks, *Sphyrnidae* family, are among charismatic aquatic animals in ocean and sea inshore. In this family, *Sphyrna lewini* as a scalloped hammerhead shark is a seasonally migratory shark and can be found worldwide in warm/tropical sea/ocean waters, such as the 'Persian Gulf' in the south of Iran. Their maximum total body length can reach 3.7-4.2 m at maturity (Copmpagno et al., 2005). In general, hammerhead shark is among agile swimmers in its habitat including ocean and sea inshore from the surface to deep waters (deeper than 275 m). Their swimming speed is about 1 m/s at cruise and can reach about 10 m/s at burst swimming (Ketchum et al., 2014). These species can be simply identified by the special shape of their head, i.e., cephalofoil, resembling a hammer-shape (Figure 1). The abovementioned unique morphology provides a higher level of stereoscopic vision (Copmpagno et al., 2005; McComb et al., 2009; Kuznar, 2017), olfactory (Kajiura et al., 2005; Kuznar, 2017) and electro-sensory capabilities (Kuznar, 2017) for the aquatic animal. Researchers have also studied the functionality of cephalofoil in the enhanced manoeuvrability of hammerhead sharks (Kajiura et al., 2003; Gaylord et al., 2020).

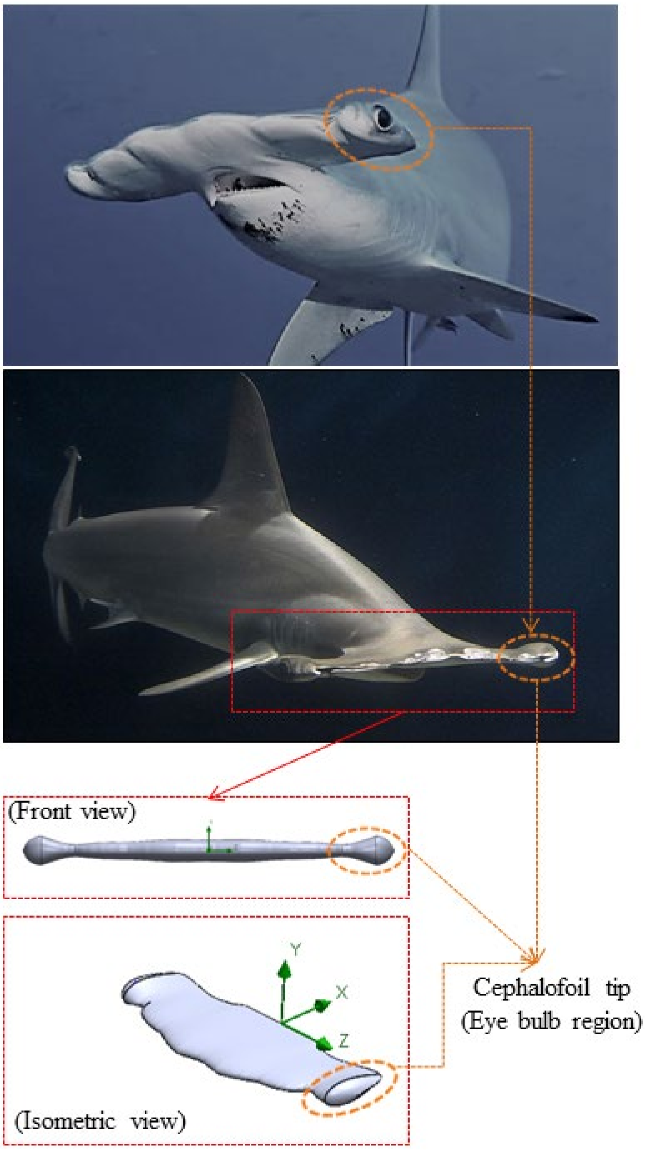

Figure 1. Geometry of the cephalofoil tip (eye bulb region) of a scalloped hammerhead shark, *S. lewini* [real geometry: top (source: twofishdivers) & middle (source: Suneko, Flickr); bottom: 3D modelled geometry]

In the present study, the evolution and characteristics of the 'tip vortex' induced by the special morphology of the cephalofoil tip of a scalloped hammerhead shark are studied (Figure 1). Our results indicate a 'winglet-like' behaviour for the cephalofoil tip of the hammerhead shark.

Materials and Methods

Computational fluid dynamics (CFD) is adopted here to study flow passing around the cephalofoil of a scalloped hammerhead shark, *S. lewini*. In general, numerical flow simulations can provide a comprehensive and more complete data set to investigate details of the flow field without limitations of the experimental techniques. In principle, CFD simulations can behave as a platform or numerical 'test rig' to study fluid flow phenomena in details and from different angles, which can be extremely helpful to grasp underlying mechanisms involved in the physics of the problem. Here, the comparative CFD simulation technique, as a powerful method in the field of bionic designs and Biomimetics, is utilized to explore the effects of the external morphology of the cephalofoil tip on the 'tip vortex' characteristics.

Geometry Models of Hammerhead Shark's Cephalofoil

Two geometries are designed to observe the hydrodynamic effects of the cephalofoil tip. The first model resembles the real geometry of the hammerhead shark's cephalofoil with tip bump, basically generated by the presence of the eye bulb (Figure 2, part A). Another geometric model is a modified version, identical to the first model except for the tip region, which is flat here without the abovementioned bump (Figure 2, part B). To construct a 3D model of the cephalofoil (Figure 2, part A), hydrofoil ribs of a scaled-down cephalofoil model are scanned and digitalized in some selected planes perpendicular to the lateral (z) axis. Overall, a total number of 13 cross-sections and 2 guide curves representing L.E. and T.E. of the cephalofoil (in the plane defined as y=0) are generated in MATLAB with high resolution and imported into the SolidWorks CAD environment. Then, the cephalofoil model is constructed by applying a so-called 'Lofting process'. The aforementioned process is performed by stitching the successive rib-section curves one by one, controlled by 2 guide curves.

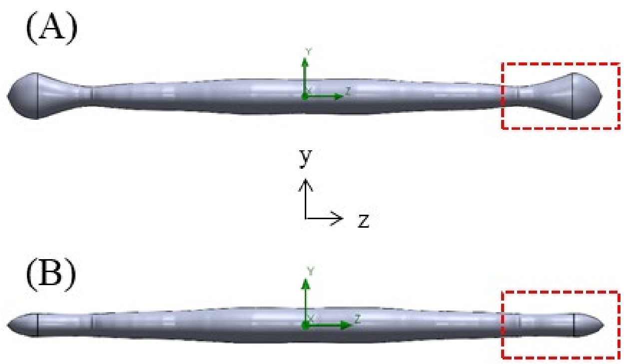

Figure 2. Adopted 3D cephalofoil geometry for a scalloped hammerhead shark: A) Real cephalofoil- geometry with a tip bump (eye bulb); B) Modified cephalofoil- imaginary geometry with a flat tip, i.e., without the tip bump (eye bulb)

Numerical Methodology

For upcoming simulations, inflow velocity is set based on a prescribed Reynolds number, i.e., 10^6, corresponding to the swimming of a juvenile hammerhead shark at low swimming speed mode with a velocity equal to 1 m/s. Water current in the aquatic environment is another factor that contributes to the calculations. In general, ocean currents vary widely geographically. As an instance, in western North Atlantic Ocean, fastest current speed is about 2.5 m/s near the ocean surface and its gulf current speed is equal to 1.8 m/s. A combination of the swimming speed with ocean and sea currents generates a wide range of angles of attack (AoA) and sideslip angles for the hammerhead shark. It is also worth mentioning that adult scalloped hammerhead sharks spend the majority of their time swimming at a rolled angle (Royer et al., 2020) to reduce transports costs (Payne et al., 2016). Figure 3 shows the coordinate system adopted to define AoA (α) and sideslip angle (ψ) for all upcoming simulations. In this regard, the shark's body and its cephalofoil are placed in a fixed position in space and the aforementioned angles α and ψ are defined by a blowing angle relative to the streamwise (x) direction. In the planned computation campaign here, a total number of 20 simulations at different AoAs and a sideslip angle are performed.

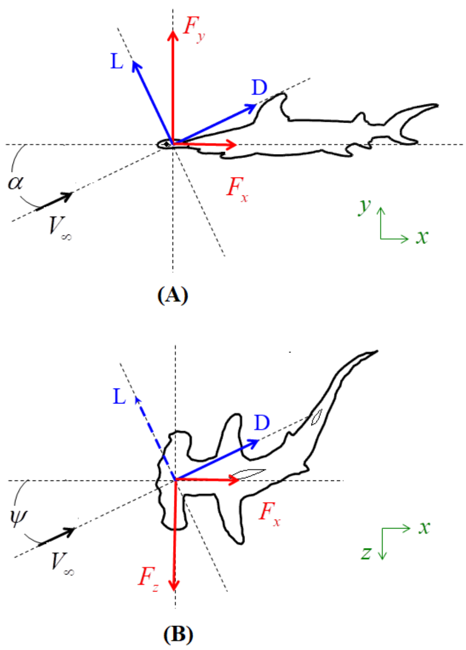

Figure 3. Coordinate system adopted for turbulent flow simulations over the cephalofoil; A) angle of attack (AoA), B) sideslip angle

Computational Domain and Grid Generation

The computational domain is considered as a cube around the cephalofoil. To minimize the boundary effects, it extends 2.5 times of the cephalofoil span (*b*) in y^- and y^+ vertical directions; 2 and 3 times of cephalofoil span in x^- and x^+ streamwise directions, respectively and 2 times of cephalofoil span in z^- and z^+ lateral directions (Figure 4). A larger extension in x^+ direction allows capturing tip vortices in full size in the computational domain. SolidWorks meshing tool with an adaptive mesh capability is adopted in the present study to construct a computational grid around the cephalofoil. After a mesh convergence test, a well-converged grid with a maximum of 2 million elements is adopted for all upcoming simulations. As one can see in Figure 4, through three levels of mesh refinements a smooth transition between the coarse mesh in the outer-flow region and fine mesh in the near-body zone is obtained. It is visible that the adaptive mesh can properly capture all fine features of the cephalofoil geometry and its corresponding boundary layers.

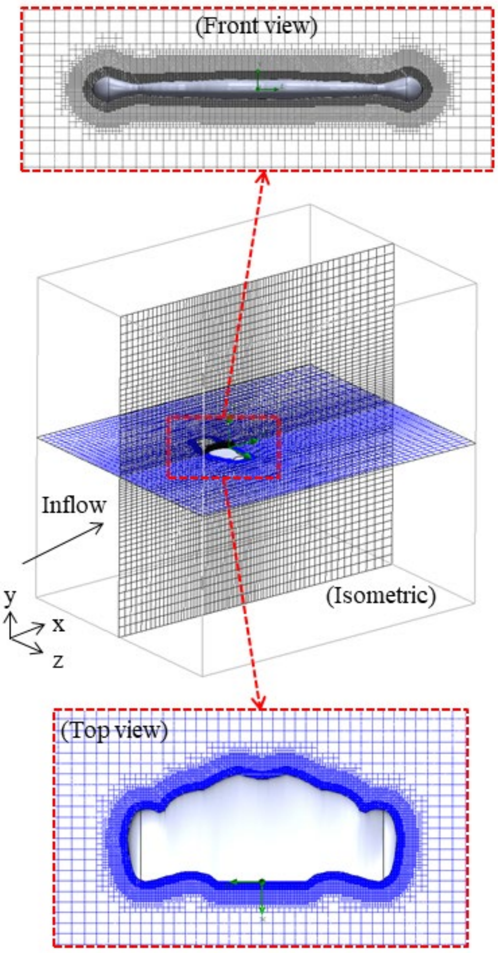

Figure 4. Computational domain with an adaptive grid generation around the cephalofoil geometry of *S. lewini*

Flow Solver & Turbulence Treatment

Incompressible Navier-Stokes equations are the governing equations of turbulent flows passing over the hammerhead shark's cephalofoil. Turbulence at high Reynolds number, $Re \sim 10^6$, is treated here using the Lam-Bremhorst turbulence model (Lam & Bremhorst, 1981) by SolidWorks Flow Simulation (SFS) solver. The solver utilizes the finite volume method along with the URANS-SIMPLE technique to obtain numerical solutions of the flow fields. In this regard, multigrid, conjugate gradient and operator-splitting techniques are also adopted for an efficient computing performance (Sobachkin & Dumnov, 2013).

For upcoming numerical flow simulations, 'inflow' is set as a prescribed inlet velocity with a blowing angle, as already explained (Figure 3). All other boundaries are set as 'outflow' in the solver. For the calculations, inflow turbulence intensity and length scale are set as 0.1% and 8×10^{-4}, respectively. SFS solver applies a rigorous statistical strategy based on a dynamic calculation of dispersion of flow variables like velocity, pressure, etc. to ensure reaching the lowest residual level for the convergence.

Cephalofoil Tip Vortex Strength

A vortex can be viewed as a relatively long-lived structure in a 3D flow field, which involves a centreline (vortex core) and a border. This entity makes the fluid particles rotate around its centreline in a circular/spiral pattern. To quantify the strength of a vortex, one should see how much powerful the vortex is to make the adjacent fluid particles rotate around its centreline. In this regard, the concept of 'circulation' can be adopted as a practical measure to quantify the strength of a vortex. Circulation (Γ) around a closed curve C in the flow field (here around the vortex core) is defined as below:

$$\Gamma = \oint_C \vec{u}.d\vec{x} = \int_A \vec{\omega}.\vec{n}\, dA \quad (1)$$

In the above equation, $\vec{u}$ and $\vec{\omega}$ denote velocity and vorticity vectors, respectively. In addition, $d\vec{x}$ stands for differential line element vector along the curve C and $\vec{n}$ is the unit vector perpendicular to the cross-sectional area A (surrounded by the closed curve C).

In general, tip vortices are produced by moving a finite wing in a fluid environment, due to spanwise flow motion which rolls up from the bottom of the wing (high pressure region) to the upper surface of the wing (low pressure region) around the wing tip. This lateral fluid flow motion superimposed with the freestream bulk flow produces a spiral vortical structure originating from the wing tip, namely a 'tip vortex'. The formation of this kind of vortices leads to an energy loss for the wing flow system, and ultimately increases the 'induced drag' of the wing. Wingtip devices are typically utilized to diminish or minimize the effects of tip vortices via controlling/suppressing tip vortex formation, like: winglets, sharklets and wingtip fences. In general, wings with winglets generate shorter and weaker tip vortex, compared to the wings without winglets.

By moving in the streamwise direction and getting farther from the wing tip, the vortex core diffuses in the lateral directions and circulation (strength of vortex) reduces under the effects of viscosity. 'Kevin's circulation theorem' describes the evolution of circulation in a flow field, here for an incompressible flow field. Mathematically 'Kevin circulation theorem' can be written as below:

$$\frac{D\Gamma}{Dt} = \underbrace{-\oint_C \frac{dp}{\rho}}_{barotropic\ term} - \underbrace{\oint_C \nu(\vec{\nabla} \times \vec{\omega}).d\vec{x}}_{diffusive/viscosity\ effects} \quad (2)$$

Where $\frac{D}{Dt}$ denotes a material temporal derivative operator. p and $\rho$are pressure and density, respectively. ν also represents kinematic viscosity. The first term on the RHS of Eq. (2) is negligible for most cases, except for barotropic flows. For our application here, i.e., flow over the cephalofoil of a hammerhead shark, the first term is omitted. The second term on the RHS of the equation indicates the effects of viscosity, as a monotonic decrease in circulation. In other words, the diffusive characteristics of viscosity tends to spread the vortex in the lateral direction towards its border and to decrease circulation quantity by getting farther and farther from the cephalofoil tip in the streamwise direction.

In the present research, to investigate evolution of the 'tip vortex strength', surface integral of the axial vorticity ω_x, as a major part of the circulation quantity, is considered over a rectangular window (Eq. (1)). The window is defined as $\Delta z \times \Delta y = [-0.3...0.3] \times [-0.3...0.3]$around the vortex core at each streamwise position behind the cephalofoil, i.e., x/b =0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0; where $b$is the cephalofoil span. In this regard, the vortex core at each vertical plane behind the cephalofoil is tracked by an in-house developed code using image processing techniques. The vortex core determines the centre of the abovementioned rectangular window.

Validation Test Case

Before switching to comparative CFD simulations of cephalofoil geometries, the adopted numerical strategy is first applied for simulations of fluid flow over a rectangular wing with unity aspect ratio at $Re \approx 1.5 \times 10^5$, as a validation test case (Taheri, 2021a). For low-aspect ratio wings, tip vortices are getting dominant over a larger portion of the wing; in this sense, lift coefficient slope decreases by lowering aspect ratio (Houghton et al., 2015). Figure 5 exhibits a very good agreement in variations of lift coefficient for the present numerical strategy in comparison to the experimental curve (Chen et al., 2012) and theoretical curve (Lowry & Polhamus, 1957). This validates the proposed numerical strategy to simulate flow over the cephalofoil of the hammer head shark, *S. lewini*.

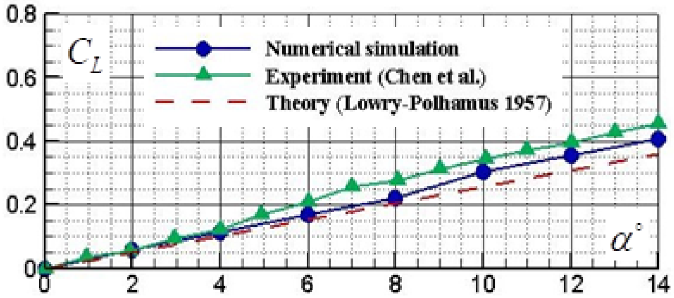

Figure 5. Lift coefficient versus AoA obtained from the adopted numerical strategy for a low-aspect ratio rectangular wing at $Re \approx 1.5 \times 10^5$

Results and Discussion

As mentioned, to study the hydrodynamic effects of cephalofoil tip eidonomy on tip vortex formation and evolution, a series of comparative turbulent flow simulations are performed. In the following sub-sections, obtained results are presented and discussed in details.

Bionic Winglet Behaviour at AoA

As discussed earlier, hammerhead sharks experience a wide range of AoAs and sideslip angles in their swimming envelope. Here, the effects of AoA variations on tip vortex evolution and characteristics are considered for the real and modified cephalofoil geometries (Figure 2). To detect the 'tip vortex' structure in the wake of a cephalofoil, different techniques can be adopted. In general, vortex detection schemes are classified into Eulerian and Lagrangian techniques; each with relative advantages and disadvantages. Basically, Eulerian techniques rely on the velocity gradient at a local point, such as λ_2-criterion. Figure 6 shows vortical structures developed over a cephalofoil for the real and modified geometries at $\alpha = 8°$ and $\alpha = 16°$, captured by $\lambda_2 = -1$. As one can see in the figure, the real cephalofoil (with tip bump) produces a shorter and weaker

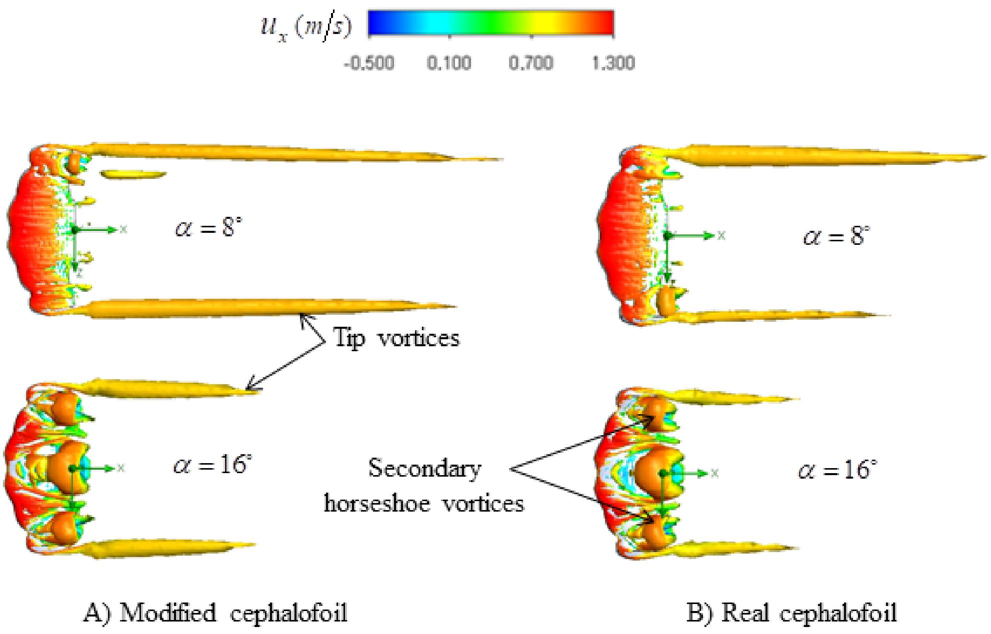

Figure 6. Tip vortices captured by λ_2-criterion ($\lambda_2 = -1$) for both real and modified cephalofoil geometries, coloured by axial velocity field

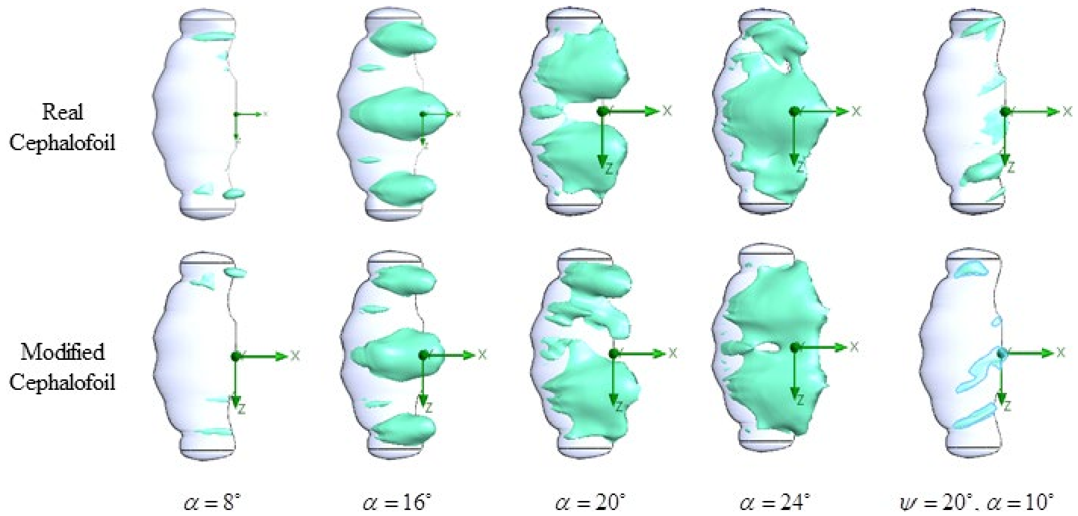

Figure 7. Flow separation zones over the real and modified geometries of the cephalofoil for different AoAs and a sideslip angle

tip vortex in its wake for both AoAs compared to the modified version. Secondary horseshoe vortices, bounded to the planform, are also generated and getting intensified by increasing AoA.

The evolution of flow separation zones (defined as, $u_x < 0$) at different AoAs and a sideslip angle is also visible in Figure 7. As one can see in the figure, for $\alpha < 16°$, the topology of uncoherent separation zones is very similar for both cephalofoil geometries, although some minor differences exist. By increasing AoA, separation zones are getting larger and finally getting merged at $\alpha \approx 24°$. For $\alpha > 16°$, separation zone topology of the real cephalofoil deviates from the modified counterpart. At sideslip angle $\psi = 20°$, separation zones are getting inclined, relative to the streamwise (x) direction. A different separation pattern forms for each version of the geometries, correlated to the wingtip eidonomy.

To visualize fluid flows passing over the cephalofoil, a tracer particle dynamic study has been performed here. In this regard, 10^3 spherical water particles with a diameter of 10^{-4} m are continuously released from the cephalofoil surface and are convected downstream by the bulk freestream flow. Ideal reflection is also applied for the fluid particle and wall interactions in the separation and recirculation zones, if any. Figure 8 indicates particle dynamics over the real cephalofoil. As one can see in the figure, primary tip vortices originating from the wing tip region and secondary vortices originating from the adjacent area of the wing tip are generated by special eidonomy of the cephalofoil. Later, more explanations about the possible effects of these weak secondary vortices are provided. In addition, counter-rotating tip vortices are captured by iso-contours of axial vorticity, as $\omega_x = \pm 1$ 1/s (Figure 8).

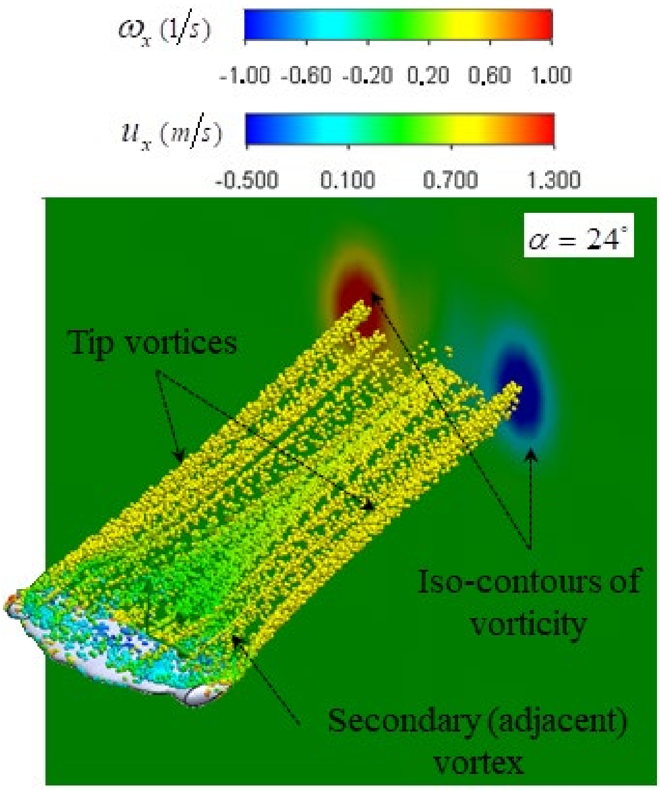

Figure 8. Tracer particle study result, superimposed with vorticity field at a downstream plane, i.e.,$x/b = 1.5$, behind the real cephalofoil at $\alpha = 24°$

To further investigate characteristics of tip vortices of the cephalofoil, the evolution of axial vorticity fields at different downstream planes, perpendicular to the streamwise (x) direction, is considered here. As an example, Figure 9 exhibits axial vorticity fields behind the cephalofoil in the rectangular window $\Delta z \times \Delta y = [-0.3...0.3] \times [-0.3...0.3]$ around the tip vortex core. The planes are placed at x/b =0.25, 0.5, 1.0, 1.5, 2.0, and 3.0; where $b$is the cephalofoil span. As one can see in the figure, at x/b =0.25 an intensive tip vortex forms along with a weaker adjacent secondary vortex. It is also visible in the figure, by getting farther downstream at a larger distance from the cephalofoil trailing edge, viscosity acts more on the vorticity field and tends to reduce the vorticity gradient in the field, in accordance to 'Kevin's circulation theorem', Eq. (2). In this way, the maximum concentrated vorticity at x/b =0.25 (visible with dark color) are getting lower and lower towards x/b =3.0. Diffusion process, implemented by viscosity, spreads the tip vortex in the lateral directions through its boundary and increases the so-called 'vortex-affected area'. In other words, vortex tip cross-section grows under the effects of viscosity, although its strength (circulation) exhibits an overall decrease by getting farther from the cephalofoil tip according to 'Kevin's theorem'. It is worth mentioning that this decrease is not always monotonic in the case of cephalofoil geometry, in the following of this sub-section the reason will be presented and discussed in details. Another important observation visible in Figure 9 from x/b =0.25 to 3.0 is the 'merger event' of the tip vortex and the adjacent secondary vortex; this process potentially contributes to the variation of circulation, as discussed shortly in the following.

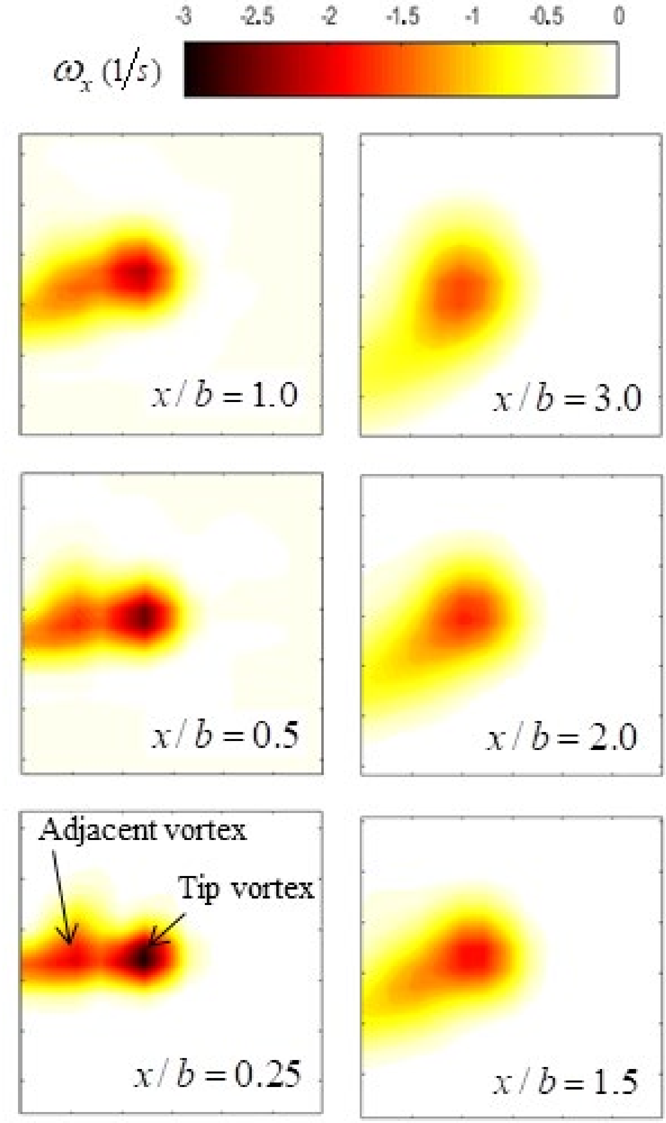

Figure 9. Evolution of tip vortex for the real cephalofoil in the streamwise direction at $\alpha = 8°$, captured by axial vorticity field

As explained before, to quantify the possible effects of the cephalofoil tip geometry on the 'tip vortex' characteristics, variation of circulation (as a measure of vortex strength) in the streamwise (x) direction is considered here. As an instance, Figure 10 shows the variation of circulation (Γ) in the streamwise direction at $\alpha = 16°$ and the maximum AoA in the present study, i.e., $\alpha = 28°$. As one can see in the figure, the circulation curve of the tip vortex for the real cephalofoil (with tip bump) is lower than one for the modified cephalofoil (without tip bump) at all streamwise positions for both AoAs. By increasing AoA, the effect is getting more pronounced. The curves demonstrate that cephalofoil bump in the eye bulb region of a hammerhead shark behaves as a winglet and produces a weaker tip vortex (with lower circulation value) compared to the version without the tip bump.

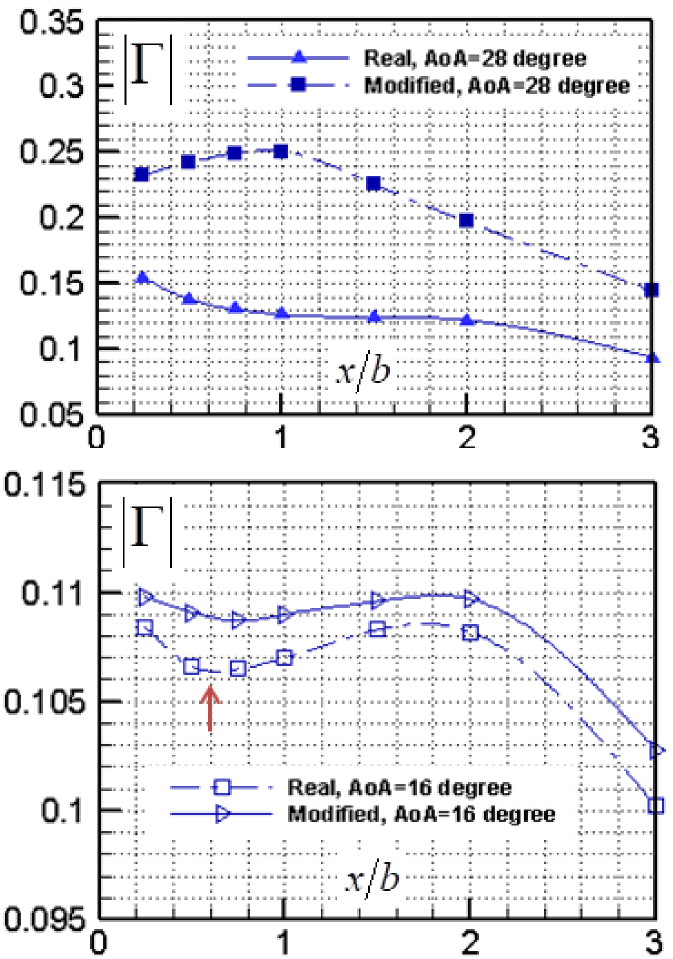

Figure 10. Variation of circulation for the real and modified cephalofoil geometries of the hammerhead shark in the streamwise direction at $\alpha = 16°$ (bottom) and $\alpha = 28°$ (top)

It is also important to notice that for an individual single vortex 'Kevin's circulation theorem' suggests that the circulation of tip vortex in streamwise direction monotonically decreases by viscosity, such as circulation curve obtained for the real cephalofoil geometry at $\alpha = 28°$ in Figure 10. On the other hand, as shown in the figure for $\alpha = 16°$, there exists an initial decrease in the circulation curve up to about $x/b = 0.6$. In the interval, $0.6 < x/b < 2.0$, circulation increases and for $x/b > 2.0$ circulation decreases again in the streamwise direction for both versions of the cephalofoil geometry. The physics responsible for the abovementioned behaviour is the 'merger phenomenon' of the tip vortex and the secondary (adjacent) vortex, as mentioned earlier (Figure 9). In fact, the monotonic decrease for the circulation in the interval $0 < x/b < 0.6$, as predicted by 'kevin's theorem' is valid for a single tip vortex. At $x/b = 0.6$ (marked by a vertical red arrow in Figure 10), tip vortex and the secondary (adjacent vortex) start to interact over the interval $0.6 < x/b < 2.0$ in a nonlinear fashion and are completely merged at around $x/b = 2.0$ and forms a stronger single vortex with higher level of circulation (strength). This process explains the observed nonlinear increase of the circulation over $0.6 < x/b < 2.0$. For $x/b > 2.0$, circulation of the single resultant tip vortex (resultant of the merger phenomenon) decreases again under the viscosity effects in a nonlinear fashion as predicted by 'Kevin's circulation theorem'. Figure 11 shows similar trends for variations of circulation of tip vortices in the streamwise direction at other simulation AoAs for the real and modified cephalofoil geometries. It is also visible in the figure that the strength of cephalofoil tip vortices gets augmented by increasing AoA.

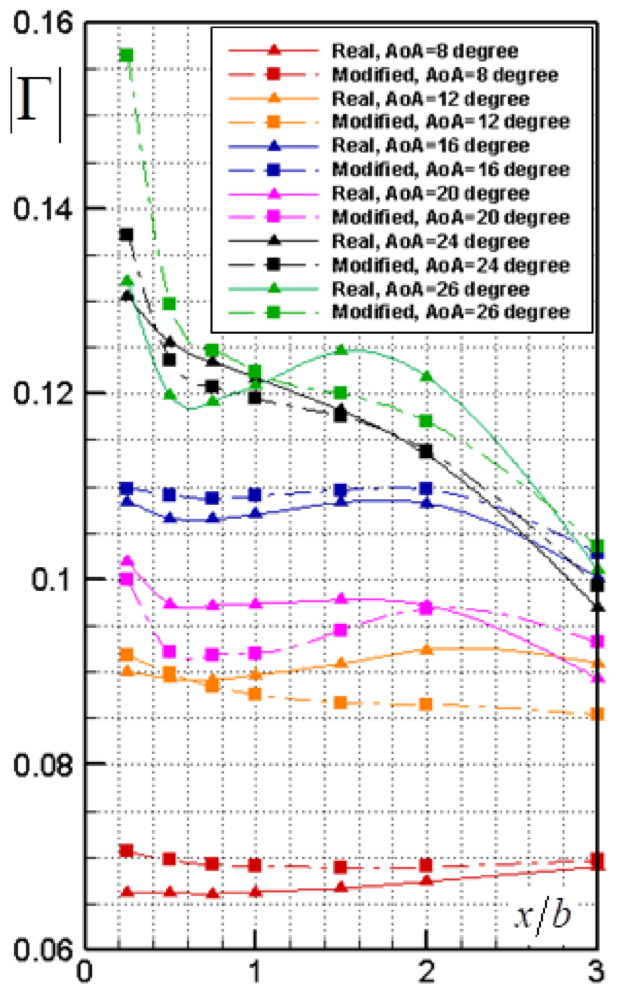

Figure 11. Variations of circulation (Γ) in the streamwise direction (x/b) for the real and modified cephalofoil geometries at all simulation AoAs

Bionic Winglet Behaviour at Sideslip Angle

As mentioned before, hammerhead sharks are agile swimmers, facing to a wide range of AoAs, sideslip and roll angles in their manoeuvres. Here in the final section, turbulent flows over both geometric versions of the cephalofoil are simulated at a sideslip angle $\psi = 20°$ along with $\alpha = 10°$. Figure 12 depicts vortical structure captured by $\lambda_2 = -1$ criterion.

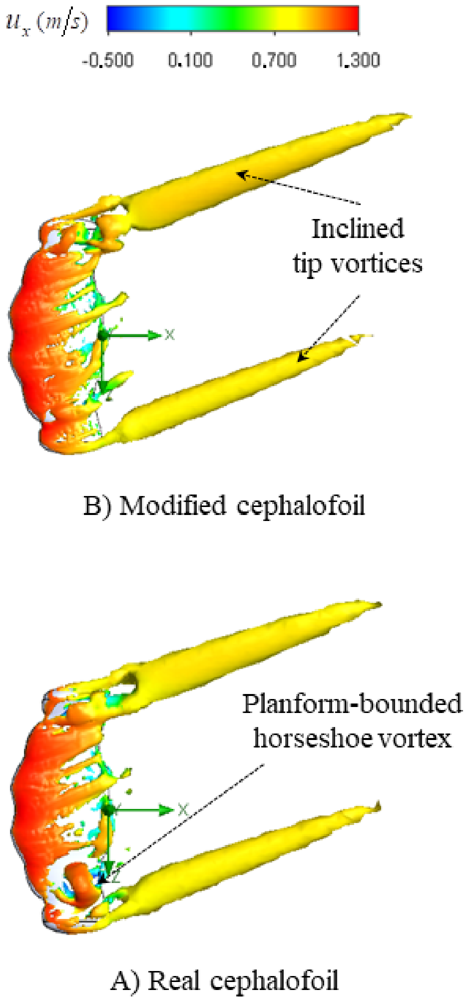

Figure 12. Vortical structure formation over the real and modified versions of the cephalofoil at sideslip angle ($\psi = 20°$ and $\alpha = 10°$)

As one can see in Figure 12, tip vortices are deflected and getting inclined due to the sideslip blowing angle $\psi = 20°$. A planform-bounded horseshoe vortex is also generated near the cephalofoil tip for the real version of the geometry, which is not present for the modified version of the geometry. Figure 13 depicts the variation of circulation in the streamwise direction for the flow simulation at the sideslip angle $\psi = 20°$. As it is visible in the figure, the circulation quantity decreases in the streamwise direction in agreement with Kevin's theorem. The real geometry of the cephalofoil behaves slightly better than the modified version at the sideslip angle, although the difference in circulation vanishes by getting farther downstream in the streamwise (x) direction.

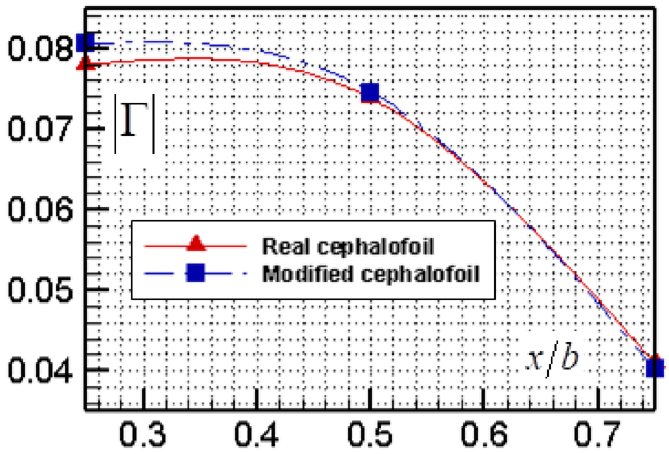

Figure 13. Variations of circulation behind the cephalofoil for both geometric versions in the streamwise direction at $\psi = 20°$ and $\alpha = 10°$

Conclusion

In the present research, it was shown that the special eidonomy of the cephalofoil tip of a scalloped hammerhead shark, *S. lewini*, exhibits winglet-type behaviour. It produces weaker and shorter tip vortices, similar to the functionality of winglets for airplanes. In fact, by special hammerhead-shape of the head and the corresponding position of its eyes, *S. lewini* takes advantages of wider ocular vision, olfactory and electro-sensory capabilities. In addition, bump at the cephalofoil tip (basically formed by the eye bulb) brings a favourable hydrodynamic characteristic for the swimming hammerhead shark by reducing the strength of tip vortices over a wide range of AoA and sideslip angles. It was also shown that the observed local increase in the circulation of the tip vortices in the streamwise direction corresponds to a nonlinear merger of the cephalofoil tip vortex and another vortical structure, originating from an adjacent region of the cephalofoil tip. Following a nonlinear transition period (after the merger event), a monotonic decrease in the circulation is restored, as expected by 'Kevin's circulation theorem' under the effects of viscosity. As turbulent flow simulations suggest, winglet effects of the cephalofoil tip decrease at sideslip angles.

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Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Bang, K., Kim, J., Lee, S. I., & Choi, H. (2016). Hydrodynamic role of longitudinal dorsal ridges in a leatherback turtle swimming. *Scientific Reports*, *6*, 34283. https://doi.org/10.1038/srep34283

Battista, N. A. (2020). Swimming through parameter subspaces of a simple anguilliform swimmer. *Integrative and Comparative Biology*, *60*(5), 1221–1235, https://doi.org/10.1093/icb/icaa130

Borazjani, I. (2008). Numerical simulations of fluid-structure interaction problems in biological flows. [Ph.D. Thesis. University of Minnesota].

Chen, J. H., Li, S. S., & Nguyen, V. T. (2012). The effect of leading edge protuberances on the performance of small aspect ratio foils. *Proceedings of the 15th International Symposium on Flow Visualization*, Belarus, pp. 1-16.

Copmpagno, L., Dando, M., & Fowler, S. (2005). *Sharks of the world* (1st ed.). Princeton University Press.

Domel, A. G., Saadat, M., Weaver, J. C., Haj-Hariri, H., Bertoldi, K., & Lauder, G. V. (2018). Shark skin-inspired designs that improve aerodynamic performance. *Journal of Royal Society Interface*, *15*, 1-9. https://doi.org/10.1098/rsif.2017.0828

Fish, F. E., Schreiber, C. M., Moored, K. W., Liu, G., Dong, H., & Bart-Smith, H. (2016). Hydrodynamic performance of aquatic flapping: efficiency of underwater flight in the manta. *Aerospace Journal*, *3*(20), 1-24. https://doi.org/10.3390/aerospace3030020

Gaylord, M. K., Blades, E. L., & Parsons, G. R. (2020). A hydrodynamics assessment of the hammerhead shark cephalofoil. *Scientific Reports*, *10*, 14495. https://doi.org/10.1038/s41598-020-71472-2

Houghton, E. L., Carpenter, P. W., Collicott, S. H., & Valentine, D. T. (2015). *Aerodynamics for Engineering Students* (Seventh Edition). Elsevier Ltd. Publication.

Kajiura, S. M., Forni, J. B., & Summers, A. P. (2003). Maneuvering in juvenile carcharhinid and sphyrnid sharks: the role of the hammerhead shark cephalofoil. *Zoology*, *106*, 19–28. https://doi.org/10.1078/0944-2006-00086

Kajiura, S. M., Forni, J. B., & Summers, A. P. (2005). Olfactory morphology of carcharhinid and sphyrnid sharks: Does the cephalofoil confer a sensory advantage?. *Journal of Morphology*, *264*, 253-263. https://doi.org/10.1002/jmor.10208

Ketchum, J. T., Hearn, A., Klimley, A. P., Espinoza, E., Peñaherrera, C., & Largier, J. L. (2014). Seasonal changes in movements and habitat preferences of the scalloped hammerhead shark (*Sphyrna lewini*) while refuging near an oceanic island. *Marine Biology*, *161*, 755–767. https://doi.org/10.1007/s00227-013-2375-5

Kuznar, Sh. (2017). Morphological variation in olfactory, optic, and electrosensory structure of juvenile scalloped hammerhead sharks (*Sphyrna lewini*) [Master Thesis. Purdue University].

Lam, C. K. G., & Bremhorst, K. A. (1981). A modified form of the $k - \varepsilon$ model for predicting wall turbulence. *Journal of Fluid Engineering*, *103*, 456–460. https://doi.org/10.1115/1.3240815

Lowry, J. G., & Polhamus, E. C. (1957). *A method for predicting lift increments due to flap deflection at low angles of attack in incompressible flow*. Report, University of North Texas Libraries.

McComb, D. M., Tricas, T. C., & Kajiura, S. M. (2009). Enhanced visual fields in hammerhead sharks. *Journal of Experimental Biology*, *212*, 4010-4018. https://doi.org/10.1242/jeb.032615

Miklosovic, D. S., Murray, M. M., Howle, L. E., & Fish, F. E. (2004). Leading-edge tubercles delay stall on humpback whale (*Megaptera novaeangliae*) flippers. *Physics of Fluids*, *16*, 39-42. https://doi.org/10.1063/1.1688341

Miles, J. G., & Battista, N. A. (2019). Naut your everyday jellyfish model: exploring how tentacles and oral arms impact locomotion. *Fluids Journal*, *4*(169), 1-43. https://doi.org/10.3390/fluids4030169

Payne, N. L., Iosilevskii, G., Barnett, A., Fischer, C., Graham, R. T., Gleiss, A. C., & Watanabe, Y. Y. (2016). Great hammerhead sharks swim on their side to reduce transports costs. *Nature Communication*, *7*(12289), 1-5. https://doi.org/10.1038/ncomms12289

Royer, M., Maloney, K., Meyer, C., Cardona, E., Payne, N., Whittingham, K., Silva, G., Blandino, C., & Holland, K. (2020). Scalloped hammerhead sharks swim on their side with diel shifts in roll magnitude and periodicity. *Animal Biotelemetry*, *8*, 11. https://doi.org/10.1186/s40317-020-00196-x

Sobachkin, A., & Dumnov, G. (2013). Numerical basis of CAD-Embedded CFD. *Proceedings of the NAFEMS World Congress*, Austria, pp. 1-19.

Taheri, A. (2018a). Lagrangian coherent structure analysis of jellyfish swimming using immersed boundary FSI simulations. *Journal of Mechanical and Civil Engineering*, *15*(1), 69-74. https://doi.org/10.9790/1684-1501046974

Taheri, A. (2018b). A meta-model for tubercle design of wing planforms inspired by humpback whale flippers. *International Journal of Aerospace and Mechanical Engineering*, *12*(3), 315-328. https://doi.org/10.5281/zendoo.1317268

Taheri, A. (2018c). On the hydrodynamic effects of humpback whale's ventral pleats. *American Journal of Fluid Dynamics*, *8*(2), 47-62. https://doi.org/10.5923/j.ajfd.20180802.02

Taheri, A. (2020). Hydrodynamic impacts of prominent longitudinal ridges on the 'whale shark' swimming. *Research in Zoology*, *10*(1), 18-30. https://doi.org/10.5923/j.zoology.20201001.03

Taheri, A. (2021a). *Fluid dynamics and bio-propulsion of animal swimming in nature: Bionics* (1st ed.). Arshadan Publication.

Taheri, A. (2021b). Lagrangian flow skeletons captured in the wake of a swimming nematode *C. elegans* using an immersed boundary fluid-structure interaction approach. *International Journal of Bioengineering and Life Sciences, 15*(7), 71-78.

Taheri, A. (2021c). Hydrodynamic analysis of bionic chimerical wing planforms inspired by manta ray eidonomy. *Indonesian Journal of Engineering and Science, 2*(3), 11-28. https://doi.org/10.51630/ijes.v2i3.25

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REVIEW ARTICLE

Black soldier fly (*Hermetia illucens*) larvae as an ecological, immune booster and economical feedstuff for aquaculture

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ABSTRACT

Black soldier fly larva can renovate biological trashes into valuable nutrients, for instance, proteins, lipids, and chitin, which decrease ecological encumbrance happening due to organic wastes accumulation. Even though rapid demand for proteinaceous food is predictable, insects got less attention in the animal feed business primarily due to technical and monetary hurdles. Moreover, many times research highlighted the consumer and producer preferences for insects' meal potential in livestock feeding. This review is anticipated to elucidate the prominence of black soldier fly larvae meal as a substitute to conventional feedstuffs including soybean and fishmeal and soybean oil ensuring productive, cost proficient, environmentally friendly, least land necessitating, least pathogenic risk, immunity-boosting, purely organic and everlasting source of non-conventional protein feedstuff for aquatic habitats.

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Introduction

Aquaculture is the most rapidly developing commerce globally. Approximately half of the global food demand is met by fish production and it will increase up to 70% until 2030 (Obiero et al., 2019). Aquaculture has been increasing widely since the last decade (FAO, 2016). Aquaculture comprises a variety of species and farming approaches, succeeding in various communal, financial, nutritive, and eco-friendly aftermaths (Gephart et al., 2020). The evolution of worldwide aquaculture upsurges the ultimatum for aqua feedstuffs. Feeds delivers nutrient to fish for quicker development, existence and support their vigorous lifespan. It represents the key of the entire operative budget in aquaculture approximately 60%

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(Pauly & Zeller, 2017). The global manufacturing of aquaculture feed was about 0.04 billion tons in 2018 (Mo et al., 2018). There is a necessity of alternative protein resources for fish feeding because fish and soybean are also the staple food of human. Moreover, poisonous gas emissions and land erosions occur due to soybean cultivation (Cottrell et al., 2020). Almost 12% of the 0.171 billion tons of entire fish production was used to produce fishmeal and fish oil in 2016 (Gerber et al., 2013). The monitoring scheme varies commonly amongst countries globally about the use of insect feeding to animals and consumer preferences and acceptance about insect utilization (Lahteenmaki-Uutela et al., 2017). In 2001, (EC 999/2001) because of Bovine Spongiform Encephalopathy (BSE), processed animal proteins (PAPs) were excluded due to contagious BSE persepective (European Parliament and Council, 2009). In the following year (European Parliament and the Council of the EU, 2002), the list of feedstuffs intended for animal feeding were outlined along with their maximum tolerable inclusion levels (Verstraete, 2013).

On 16 January 2013, Annexes I and IV to EC Regulation No: 999/2001 was amended along with directive 2002/32/EC and EC Regulation No: 56/2013 acknowledged the use of non-ruminant originated processed animal proteins to be permissible in aquaculture (European Parliament, 2013). Nonetheless, insects were not specifically enclosed in the raw material directory of the European Commission. So, on 24 May 2017, EC amended annexes X, XIV and XV to EU Regulation No: 142/2011 and Annexes I and IV to EC regulation No: 999/2001 and EU Regulation No: 2017/893 was publicized whose Annex II specifically stated the farmed insect derived proteins to be utilized for the aquaculture, farmed animals other than fur animals and pet animals (European Commission, 2017) and it allowed use of three species of Crickets including *Gryllus assimilis, Acheta domesticus* and *Gryllodes sigillatus*; two species of Mealworms including *Alphitobius diaperinus* and *Tenebrio molitor*; common house fly (*Musca domestica*) and black soldier fly (*Hermetia illucens*). Moreover, in this regulation the fat extracted from these insects was allowed to be fed to every sort of animal. Annex III to Regulation No: 767/2009 forbids the use of feces and digestive tract content although these ingredients are used in other countries for insect production. Rendering to EC Regulation No: 1069/2009 broadcasted on 21 October 2009, insects are well-thought-out as 'farmed animals' and therefore, for their feeding the use of dung, kitchen waste comprising meat and fish, are not permissible (European Parliament and Council, 2010).

The present review illustrates the importance of black soldier fly larva in aquaculture feeding as an economical, more environmentally friendly, immunizing and productive ingredient.

Black Soldier Fly (BSF) as Eco-Friendly Feed Ingredient

Feed and food demand puts a key environmental impact worldwide that could be increased up to 80% by 2050 due to the nonexistence of high-tech modification and alleviation processes (Wiseman et al., 2019). Livestock is considered to be the most environmentally detrimental of all the evolutionary happenings with immense primary and secondary influences to global warming (75% of total agricultural emissions) (Springmann et al., 2018). Owing to less feed efficacies, poor intestinal fermentation in cattle, and higher gaseous emanation in feces there is extensive ecological humiliation as land deprivation, harm to natural habitats, greenhouse effects, and stress on aquatic and land species (Sakadevan & Nguyen, 2017). Restaurant, bakery and kitchen waste is getting increased consideration for its management due to its undesirable ecological, social and health influences (Teigiserova et al., 2020). Undeniably, the EU expected this Sort of waste material volumes to about 100 million tons per year in the European Union countries recommending that the forecast for 2020 is 140 million tons (Guo et al., 2021). According to the European Union Waste Framework Directive (2008) efficient utilization of such sort of organic wastes must be the foremost stratagem to decline the ecological hazards in Europe.

Keeping this in view, the environmental responsiveness of insects as food and feed has been studied widely with orientation to alternatively sustainable, ecologically and economically sufficient protein sources (Gahukar, 2016). Biotransformation of BSF larvae and contribution to the greenhouse emissions could be changed according to the diversity and availability of the substrates (Rahmi et al., 2020). The feeding of bio-wastes to BSF has direct effect on the larvae growth performance, waste conversion ratio, and nutritional contents of BSF larvae (Raksasat et al., 2020). In comparative research conducted about putrefaction of organic wastes by BSF larvae versus aerobic bacteria by Perednia et al. (2017), it was reported that larvae consumed the waste material within one week with 28% of CO_2 emission while bacterial decomposers utilized it within 45 days with 50% CO_2 discharge in the environment. It was reported that the BSF larvae converted about 45% of the biomass fed into valuable feedstuff enriched with protein, fats and chitin. In research conducted about BSF larvae growth on restaurant waste and vegetable/fruit waste, it was determined that CH_4 and N_2O emissions were very low as compared to the conventional protein feedstuffs (Bosch et al., 2019). According to Bosch et al. (2019), the impact of BSF larvae on environmental pollution was relatively very low as compared to the soybean and fishmeal when BSF was grown on manure and food waste. Rearing of BSF larvae by using bovine manure and other metropolitan waste emits lower greenhouse gases rather than using beet pulp as a substrate for larvae growth. Similarly, larvae have grown on municipal wastes and has emitted 2.1 kg of CO_2 and 0.05 m^2 land has been used per kg of larval protein (Salomone et al., 2017). Moreover, it was reported that there is no significant conservational influence when animal feedstuffs like sorghum, DDGs, beet pulp, poultry feed is being used for BSF rearing. It was scientifically evaluated in a research trial where BSF larvae was grown on manure containing an antibiotic, tetracycline residues (Caligiani et al., 2018) and insecticides (Lalander et al., 2016) and it significantly degraded these residues, thus it could be stated that BSF production assists to eliminate the ecological hazards and public health concerns.

BSF as Immunity Booster Ingredient

BSF larvae have immune regulating properties due to some of antimicrobial peptides and chitin present in them. In a study, broiler chicks were confronted orally with *Salmonella* and *Escherichia coli* and fed with mealworm as a substitute to antibiotics, as a result, the cecal contents of *Salmonella* and *E. coli* were decreased along with the rising level of serum IgG and IgA and it was indorsed due to the effectiveness of chitin content of insects acting as probiotic (Islam & Yang, 2017). In a study conducted on chick manure, house fly larvae reduced the levels of *E. coli*, *Salmonella enteritidis* and *Campylobacter jejuni* (Nordentoft et al., 2017). Similarly, black soldier fly larvae have been reported to reduce *E. coli O157:H7* and *S. enterica* serovar *enteritidis* in poultry manure (Erickson et al., 2004). Research on broilers show that feeding BSF larvae initiates immune response against *Salmonella enterica* serovar *Gallinarum,* a typhoid fever causing strain in chicks. (Provost et al., 2011). Moreover, using 0.2% chitosan obtained from BSF larvae reduced the number of *S. enterica* serovar *Typhimurium* in broilers (Menconi et al., 2014).

A phenolic compound, melanin, is a bio-active component that is used as a feed additive to prevent stress and tumors in farmed animals (Cordero & Casadevall, 2020). Melanin that imparts black color to black soldier fly is eumelanin and it has a wide range of antibacterial and antifungal action (Ushakova et al., 2017). In a study, the extract of BSF larvae was evaluated for antibacterial action by the agar diffusion method. The concentration of 320 mg/ml showed a potent ($P<0.05$) bactericidal action against *Salmonella* and *E. coli* due to the presence of chitin and 49.5% lauric acid, a strong antibacterial saturated fatty acid (Harlystiarini et al., 2019). Similarly, methanol extract of BSF larvae has shown a reduction in growth and proliferation of gram-negative bacteria including *Neisseria gonorrhoeae, Klebsiella pneumoniae, Shigella spp.* (Choi et al., 2012). Not only BSF larvae show activity against gram-negative bacteria but also against gram-positive bacteria. A new defensin-like peptide 4 (DLP4) was harvested from the hemolymph of BSF larvae and it was purified by chromatography and resulted to have strong antibacterial activity against MRSA (methicillin resistant *staphylococcus aureus*) (Park et al., 2015). Similar to this, cecropin-like peptide 1 (CLP1) was extracted and purified from the hemolymph of BSF larvae and evaluated to have good inhibitory activity against gram-negative bacteria (Park & Yoe, 2017). Anti- *H. pylori* peptides were extracted from the hemolymph of BSF larvae and were used to combat metronidazole resistance against these bacteria (Alvarez et al., 2019). In a study, two antimicrobial peptides extracted from BSF larvae termed as Hidefensin-1, Hidiptericin-1 decreased the growth of *Streptococcus pneumoniae* and *Escherichia coli* by bacterial membrane lysing (Xu et al., 2020). The lyophilized BSF larvae methanol extract was evaluated to have good action against *Staphylococcus aureus* and *pseudomonas aeruginosa*. After HPLC, the extract showed a strong activity against methicillin resistant *staphylococcus aureus* (MRSA) (Park et al., 2014). Hexanedioic acid or apidic acid from BSF has shown growth inhibition effects against *MRSA*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Shigella dysenteriae* in dose dependent manner (Choi & Jiang, 2014). Recombinant attacin-like peptide obtained from BSF has shown bacteriostatic activity against *MRSA* and *E. coli* (Shin & Park, 2019).

Based upon above-mentioned studies it is clear that BSF larvae have many sorts of antimicrobial peptides that prevent the animals from diseases and averts the use of antibiotics in feed and certainly discourage antibiotic resistance.

BSF Production and Larvae Defattening Techniques

For the production of BSF larvae, there are some optimum parameters that should be controlled for their proper BSF breeding and larva hatching. The nutritional profile varies with respect to the substrate used for larvae rearing. For instance, in a study aiming to research breeding techniques of BSF, it was shown that the best larvae density was 2 larva/gram of the feed and optimum oviposition was 8500 individuals/m^3 under a maximum of sunlight exposure. The prepupae provided with soybean meal, euphorbia leaves supplemented with colza oil were enriched in C18:2n-6 fatty acid, a fatty acid essential for the growth of tilapia fish. The prepupae provided with soybean meal and fish offal were enriched in C20:5n-3, a fatty acid healthy for humans. Moreover, for optimum production, a temperature of 26.7±0.49°C and light duration of not less than 12 hours is required. The cost of larva production using soybean and fish offal was 1.83 USD per kg of the larvae (Gougbedji et al., 2021). According to Bekker et al. (2021), the effects of moisture content in the substrates was evaluated on the growth rate of the larvae and the results showed highest growth efficiency (0.62) at 45% moisture and lowest (0.52) at 75% moisture content. BSFL larvae have approximately 30% fat as dry matter basis. High fat level causes the product prone to lipid peroxidation making unpleasant texture and flavor. Moreover, it may cause smearing and blocking of the pelleting machines. A simple and competent method to defat BSF larvae is the mechanical extraction by a screw press as used in oil extractions of nuts and seeds. At 100°C the fat is squeezed from the larvae by screw press giving cake and liquid. Further processing separates protein meal from the cake and BSF larvae fat from the press liquid (Matthäus et al., 2019). Another way to extract fat from the BSF larvae is microwave assisted solvent extraction method. In this method, first of all larvae cell disruption is performed by a rotor-stator homogenizer. Then, the insect slurry is dried in a thermal dryer. Then the dried insect powders (about 20 g) are placed in holder with hexane solvent. This lipid extraction process is performed using a microwave equipment. The liquid part consisting of lipids and solvent, and residual defatted insect powders are physically divided after lipid extraction by centrifugation (Feng et al., 2019). According to 2-methyloxolane (2-MeO) has better lipid extraction capability, improved bioactivity in the BSF oil, and comparatively superior protein quality in the defatted flour as compared to n-hexane and hence it is the best solvent for BSF larvae fractionation (Ravi et al., 2019). In another study, organic solvents were compared with inorganic solvent and the results showed that organic acid increase the lipid extraction yield as 45% in case of organic solvent that was 35% in case of inorganic solvents. Organic lactic acid increased the lipid purity from 75% to 85% with 60% of protein purity (Soetemans et al., 2019). Many protease enzymes are used for lipid extraction to fast the process in solvent extraction method. Among them, protamex is the more effective enzyme during pretreatment increasing the fat fraction 2.2 times more as compared to when no enzyme was used (Su et al., 2009). Another technique for larvae fat extraction is by using CO_2 supercritical extraction method. İn this method, 10–18 mm sized crushed larval powder is delivered to carbon dioxide supercritical extraction machine at 350 bar pressure for 6 hours and it produces a powder with maximum 5% of fat (Kim et al., 2019). Another way to extract fat from larvae is by wet mode fractionation (WMF). In this method, BSF larvae are steam blenched to obtain pulpy texture. After that BSF larvae juice and press cake is obtained. The BSF juice part is homogenized and incubated with enzymes and subsequent centrifugation gives four phases (lipid, cream, aqueous and solid residuals) having more aqueous and solid fraction. Moreover, the press cake contains 60 % of the chitin in it (Ravi et al., 2021).

Significance of BSF in Aquaculture

Insects are the most valuable feedstuffs for fish feeding and amongst the insects, the nutritional profile of the black soldier fly larva is very close to and sometimes better than fishmeal. Moreover, insects are easy to house and grow, cause an insignificant environmental influence and have high protein ratio as compared to conventional feedstuffs (Llagostera et al., 2019). Many studies signify the importance of the BSF larvae in fish farming. In a recent study, the fish meal was replaced fully and partially by BSF prepupae for climbing perch *Anabas testudineus*. The results showed that final weight and crude protein of fillets were similar to the control group while crude fat and protein efficiency ratio was better in the 100 percent replacement group (Vongvichith et al., 2020). In a 56-day trial, soybean meal was replaced with defatted BSF larvae meal and fed to juvenile grass carp and the results showed no significant difference in feed efficiency, productivity. However, triglyceride content and total antioxidant status was better in 100% BSF fed group as compared to soybean fed group (Lu et al., 2020). Defatted BSF larvae meal was replaced with fish meal and fed to Japanese seabass (*Lateolabrax japonicus*) without any significant effect on final weight, hepatic and gastric histopathology, antioxidant status and immunity. Moreover, deposition of lipids in the liver was reduced in BSF fed groups as compared to the control (Wang et al., 2019). Fish meal was replaced with BSF larvae meal and fed to juvenile rainbow trout (*Oncorhynchus mykiss*) for intestinal microbial population effect and results indicated the proliferation of lactic acid bacteria, *Megasphaera, Pectinatus, Selenomonas, Zymophilus, actinobacteria* and *Bacillaceae* (Huyben et al., 2019). Similarly, fish meal replacement was performed with BSF larvae meal for juvenile Jian carp and it was reported to have significant effect on body weight gain (Zhou et al., 2018). Partially defatted BSF larvae meal was replaced with fish meal and fed to rainbow trout (*Oncorhynchus mykiss*) without any significant difference on the growth rate, villus and crypt depth and dorsal fillet quality and higher apparent digestibility coefficient of dry matter and crude protein were seen for BSF fed groups as compared to fish meal group (Renna et al., 2017). Partially defatted BSF larvae meal was fed to rainbow trout diet as a replacement to fish meal and the results showed no difference in growth and performance while higher concentration of Hydroxyproline in BSF fed groups was seen as compared to the control group (Dumas et al., 2018). According to (Muin et al., 2017), fish meal can be substituted up to 50% with BSF larvae meal without affecting growth performance and efficient feed utilization. Fish meal and soybean meal were substituted with BSF larvae and fed to Nile tilapia without having any adverse effect on fish meat quality parameters, growth and feed efficiency (Devic et al., 2018). BSF full-fat larvae meal was used as a replacement to fishmeal and fed to zebrafish and the result showed a better lipid profile in the meat of the fish (Zarantoniello et al., 2018). Many studies have shown that 50% substitution of the fish meal with BSF larvae meal is the best conciliation between ingredient bioavailability and appropriate fish development and production (Zarantoniello et al., 2020; Oteri et al., 2021). Fish meal was replaced with BSF prepupae meal and fed to European seabass (*Dicentrarchus labrax*) juveniles and results showed that there is no significant difference in weight gain, feed utilization and plasma metabolic parameters. Moreover, the apparent digestibility coefficient of histidine, arginine and valine was more in BSF fed groups as compared to the control (Magalhães et al., 2017). Recently, European seabass (*D. labrax*) was confronted with *Vibrio alginolyticus* and fed with BSF larvae meal as a partial substitution to fishmeal and the result showed a linear significant decrease in tissue and blood malondialdehyde level and increase in superoxide dismutase, catalase and glutathione peroxidase enzyme activities. Moreover, the serum lysozymes and phagocytic actions was increased in BSF larvae fed groups as compared to the control group (Abdel-Latif et al., 2021). Fish meal was replaced with BSF larvae meal in the diet of juvenile white shrimps and results showed that there was no significant difference in weight gain and feed conversion as compared to the control group (Cummins et al., 2017). Partial replacement of fishmeal was done with BSF larvae meal and fed to rainbow trout juveniles without decreasing growth performance, feed conversion rate and product quality (Stadtlander et al., 2017). In another study, rainbow trout were fed with BSF larvae meal as compared to the fishmeal in control. The physical and chemical analysis of the dorsal fillets were performed after freezing them for 120 days. The results showed that higher levels of replacement did not alter the pH, color, shear force and water holding capacity while the monounsaturated fatty acids in the flesh were lower as compared to the control (Secci et al., 2019). Juveniles of *Argyrosomus regius* were fed with BSF larvae meal as replacement to fish meal and the results showed that saturated fatty acids and polyunsaturated fatty acids, lauric acid and plasma glucose levels were increased with respect to the increasing level of BSF larvae meal supplementation in the experimental diets as compared to the control (Guerreiro et al., 2020). In a 65-day feeding trial of BSF larvae meal as replacement to fish meal in yellow catfish showed increased growth performances and serum lysozyme action enhancing immune response as compared to the control group (Xiao et al., 2018). In another study, juveniles of African catfish (*Clarias gariepinus*) were fed with BSF larvae as a substitution to fishmeal and the results showed that the group supplemented with 50% of the BSF meal have highest body weight gain, better growth rate and nutrient utilization indexes, improved superoxide dismutase and catalase activity contributing to valuable antioxidant status as compared to the control group (Fawole et al., 2020). Nile tilapia (*Oreochromis niloticus*) juveniles were fed with BSF larvae meal as replacement to fishmeal for 3 months and the results showed that the growth parameters and feed efficiency were not significantly different ($P>0.05$) amongst all of the experimental groups. Moreover, the lysozyme and peroxidase activity were enhanced in BSF fed groups as compared to the control group (Tippayadara et al., 2021). In 42 days feeding trial of BSF larvae meal as replacement to fishmeal in juvenile barramundi (*Lates calcarifer*), the results showed no significant difference on growth but the immunity and intraperitoneal fat were improved in BSF fed group as compared to the control (Chaklader et al., 2020). There are many more studies which indicate the positive influence of BSF larvae meal in aquaculture without influencing growth and health performance.

Conclusion

Based on the above-mentioned studies, black solider fly larva is an effective replacement for fish meal and soybean meal for feeding aquaculture without any negative impact on production. The nutritional profile of BSF larvae varies with respect to organic wastes being used. Growing BSF larvae on vegetables, fruits, kitchen wastes and leftover poultry wasted feeds produce larvae having an excellent nutritional profile. Moreover, the essential amino acids contents are also good in BSF larvae as compared to expensive soybean and fish meal. Generally, the protein and fat content of BSF larvae is very equivalent to soybean and fish meal. Substitution of conventional protein ingredients don't have any negative effects on the production and product quality if used in a limited percentage. With respect to economics and environmental impact, BSF larvae are very cheaper to grow because they only need biowastes, and very little land and labor are required for BSF farming. It helps to reduce the impact of organic wastes on land and convert them to organic fertilizers. BSF larvae do not release any poisonous gases in the environment as compared to soybean. There is no uncertainty to conclude that BSF larvae production for aquaculture has the potential to decrease the feed costs and it provides supernatural, environmentally friendly, and immunity boosting feed to aquatic habitats.

Compliance With Ethical Standards

Authors' Contributions

Author SRAS has planned and written the manuscript and author ISÇ has technically studied the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Abdel-Latif, H. M., Abdel-Tawwab, M., Khalil, R. H., Metwally, A. A., Shakweer, M. S., Ghetas, H. A., & Khallaf, M. A. (2021). Black soldier fly (*Hermetia illucens*) larvae meal in diets of European seabass: Effects on antioxidative capacity, non-specific immunity, transcriptomic responses, and resistance to the challenge with *Vibrio alginolyticus*. *Fish and Shellfish Immunology*, *111*, 111-118. https://doi.org/10.1016/j.fsi.2021.01.013

Alvarez, D., Wilkinson, K. A., Treilhou, M., Castillo, D., & Sauvain, M. (2019). Prospecting peptides isolated from black soldier fly (Diptera: Stratiomyidae) with antimicrobial activity against Helicobacter pylori (Campylobacterales: Helicobacteraceae). *Journal of Insect Science*, *19*, 17. https://doi.org/10.1093/jisesa/iez120

Bekker, N. S., Heidelbach, S., Vestergaard, S. Z., Nielsen, M. E., Riisgaard-Jensen, M., Zeuner, E. J., & Eriksen, N. T. (2021). Impact of substrate moisture content on growth and metabolic performance of black soldier fly larvae. *Waste Management*, *127*, 73-79. https://doi.org/10.1016/j.wasman.2021.04.028

Bosch, G., Van Zanten, H., Zamprogna, A., Veenenbos, M., Meijer, N., Van der Fels-Klerx, H., & Van Loon, J. (2019). Conversion of organic resources by black soldier fly larvae: legislation, efficiency and environmental impact. *Journal of Cleaner Production*, *222*, 355-363. https://doi.org/10.1016/j.jclepro.2019.02.270

Caligiani, A., Marseglia, A., Leni, G., Baldassarre, S., Maistrello, L., Dossena, A., & Sforza, S. (2018). Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. *Food Research International*, *105*, 812-820. https://doi.org/10.1016/j.foodres.2017.12.012

Chaklader, M. R., Howieson, J., Fotedar, R., & Siddik, M. A. (2020). Supplementation of *Hermetia illucens* larvae in poultry by-product meal-based barramundi, lates calcarifer diets improves adipocyte cell size, skin barrier functions, and immune responses. *Frontiers in Nutrition*, *7*, 320. https://doi.org/10.3389/fnut.2020.613158

Choi, W. H., & Jiang, M. (2014). Evaluation of antibacterial activity of hexanedioic acid isolated from *Hermetia illucens* larvae. *Journal of Applied Biomedicine*, *12*, 179-189. https://doi.org/10.1016/j.jab.2014.01.003

Choi, W. H., Yun, J. H., Chu, J. P., & Chu, K. B. (2012). Antibacterial effect of extracts of *Hermetia illucens* larvae against Gram-negative bacteria. *Entomological Research*, *42*, 219-226. https://doi.org/10.1111/j.1748-5967.2012.00465.x

Cordero, R. J., & Casadevall, A. (2020). Melanin. *Current Biology*, *30*, R142-R143. https://doi.org/10.1016/j.cub.2019.12.042

Cottrell, R. S., Blanchard, J. L., Halpern, B. S., Metian, M., & Froehlich, H. E. (2020). Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030. *Nature Food*, *1*, 301-308. https://doi.org/10.1038/s43016-020-0078-x

Cummins Jr, V. C., Rawles, S. D., Thompson, K. R., Velasquez, A., Kobayashi, Y., Hager, J., & Webster, C. D. (2017). Evaluation of *Hermetia illucens* larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp. *Aquaculture*, *473*, 337 344. https://doi.org/10.1016/j.aquaculture.2017.02.022

Devic, E., Leschen, W., Murray, F., & Little, D. C. (2018). Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing Black Soldier Fly (*Hermetia illucens*) larvae meal. *Aquaculture Nutrition*, *24*, 416-423. https://doi.org/10.1111/anu.12573

Dumas, A., Raggi, T., Barkhouse, J., Lewis, E., & Weltzien, E. (2018). The oil fraction and partially defatted meal of *Hermetia illucens* affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout. *Aquaculture*, *492*, 24-34. https://doi.org/10.1016/j.aquaculture.2018.03.038

Erickson, M. C., Islam, M., Sheppard, C., Liao, J., & Doyle, M. P. (2004). Reduction of *Escherichia coli* O157: H7 and *Salmonella enterica* Serovar Enteritidis in chicken manure by larvae of the black soldier fly. *Journal of Food Protection*, *67*, 685-690. https://doi.org/10.4315/0362-028X-67.4.685

European Commission. (2010). Preparatory Study on Food Waste Across EU 27. Technical Report 2010-054. In *October*. https://www.eea.europa.eu/data-and-aps/

European Commission. (2017). Commission Regulation 2017/893 as regards to the provisions on processed animal proteins. *Official Journal of the European Union*. https://op.europa.eu/en/publication-detail/

European Parliament & Council. (2009). Regulation (EC) No 1069/2009, Animal by-products Regulation. *Official Journal of the European Union*. https://eur-lex.europa.eu/legal

European Parliament & the Council of the EU. (2002). Directive of The European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed 2002/32. *Official Journal of the European Communities*. https://www.fao.org/faolex/results/

European Parliament. (2013). Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. In *Official Journal of the European Communities*. https://www.fao.org/faolex/

European Union (2009). Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC & 2003/30/EC. *Official Journal of the European Union* *5*, 2009. https://www.legislation.gov.uk/eudr/2009/28

European Union Waste Framework Directive. (2008). Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives (Text with EEA relevance). In *Official Journal of the European Union*. https://www.legislation.gov.uk/eudr/

FAO. (2016). *The State of World Fisheries and Aquaculture: Contributing to food security and nutrition for all* (pp. 200-204). Rome. https://www.fao.org/documents/

Fawole, F. J., Adeoye, A. A., Tiamiyu, L. O., Ajala, K. I., Obadura, S. O., & Ganiyu, I. O. (2020). Substituting fishmeal with *Hermetia illucens* in the diets of African catfish: Effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker. *Aquaculture*, *518*, 734849. https://doi.org/10.1016/j.aquaculture.2019.734849

Feng, W., Xiong, H., Wang, W., Duan, X., Yang, T., Wu, C., & Wang, C. (2019). Energy consumption analysis of lipid extraction from black soldier fly biomass. *Energy*, *185*, 1076-1085. https://doi.org/10.1016/j.energy.2019.07.113

Gahukar, R. (2016). Edible insects farming: efficiency and impact on family livelihood, food security, and environment compared with livestock and crops, Insects as sustainable food ingredients (pp. 85-111). Elsevier. https://doi.org/10.1016/b978-0-12-802856-8.00004-1

Gephart, J. A., Golden, C. D., Asche, F., Belton, B., Brugere, C., Froehlich, H. E., Fry, J. P., Halpern, B. S., Hicks, C. C., & Jones, R. C. (2020). Scenarios for global aquaculture and its role in human nutrition. *Reviews in Fisheries Science and Aquaculture*, *29*, 122-138. https://doi.org/10.1080/23308249.2020.1782342

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). *Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities*. Food and Agriculture Organization of the United Nations (FAO). Rome.

Gougbedji, A., Agbohessou, P., Lalèyè, P. A., Francis, F., & Megido, R. C. (2021). Technical basis for the small-scale production of black soldier fly, *Hermetia illucens* (L. 1758), meal as fish feed in Benin. *Journal of Agriculture and Food Research, 4,* 100153. https://doi.org/10.1016/j.jafr.2021.100153

Guerreiro, I., Castro, C., Antunes, B., Coutinho, F., Rangel, F., Couto, A., Serra, C. R., Peres, H., Pousão-Ferreira, P., & Matos, E. (2020). Catching black soldier fly for meagre: Growth, whole-body fatty acid profile and metabolic responses. *Aquaculture*, *516*, 734613. https://doi.org/10.1016/j.aquaculture.2019.734613

Guo, H., Jiang, C., Zhang, Z., Lu, W., & Wang, H. (2021). Material flow analysis and life cycle assessment of food waste bioconversion by black soldier fly larvae. *Science of The Total Environment*, *750*, 141656. https://doi.org/10.1016/j.scitotenv.2020.141656

Harlystiarini, H., Mutia, R., Wibawan, I. W. T., & Astuti, D. A. (2019). In vitro antibacterial activity of black soldier fly (*Hermetia illucens*) larva extracts against gram-negative bacteria. *Buletin Peternakan*, *43*(2), 125-129. https://doi.org/10.21059/buletinpeternak.v43i2.42833

Huyben, D., Vidaković, A., Hallgren, S. W., & Langeland, M (2019). High-throughput sequencing of gut microbiota in rainbow trout fed larval and pre-pupae stages of black soldier fly. *Aquaculture*, *500*, 485-491. https://doi.org/10.1016/j.aquaculture.2018.10.034

Islam, M. M., & Yang, C. J. (2017). Efficacy of mealworm and super mealworm larvae probiotics as an alternative to antibiotics challenged orally with *Salmonella* and *E. coli* infection in broiler chicks. *Poultry Science*, *96*, 27-34. https://doi.org/10.3382/ps/pew220

Kim, S. W., Jung, T. S., Ha, Y. J., Gal, S. W., Noh, C. W., Kim, I. S., & Yoo, J. H. (2019). Removal of fat from crushed black soldier fly larvae by carbon dioxide supercritical extraction. *Journal of Animal Feed Sciences*, *28*(1), 83-88. http://doi.org/10.22358/jafs/105132/2019

Lahteenmaki-Uutela, A., Grmelová, N., Hénault-Ethier, L., Deschamps, M. -H., Vandenberg, G. W., Zhao, A., Zhang, Y., Yang, B., & Nemane, V. (2017). Insects as food and feed: laws of the European Union, United States, Canada, Mexico, Australia, and China. *European Food and Feed Law Review*, *12*(1), 22-36.

Lalander, C., Senecal, J., Calvo, M. G., Ahrens, L., Josefsson, S., Wiberg, K., & Vinnerås, B. (2016). Fate of pharmaceuticals and pesticides in fly larvae composting. *Science of The Total Environment*, *565*, 279-286. https://doi.org/10.1016/j.scitotenv.2016.04.147

Llagostera, P. F., Kallas, Z., Reig, L., & de Gea, D. A. (2019). The use of insect meal as a sustainable feeding alternative in aquaculture: Current situation, Spanish consumers' perceptions and willingness to pay. *Journal of Cleaner Production*, *229*, 10-21. https://doi.org/10.1016/j.jclepro.2019.05.012

Lu, R., Chen, Y., Yu, W., Lin, M., Yang, G., Qin, C., Meng, X., Zhang, Y., Ji, H., & Nie, G. (2020). Defatted black soldier fly (*Hermetia illucens*) larvae meal can replace soybean meal in juvenile grass carp (*Ctenopharyngodon idellus*) diets. *Aquaculture Reports*, *18*, 100520. https://doi.org/10.1016/j.aqrep.2020.100520

Magalhães, R., Sánchez-López, A., Leal, R. S., Martínez-Llorens, S., Oliva-Teles, A., & Peres, H. (2017). Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). *Aquaculture*, *476*, 79-85. https://doi.org/10.1016/j.aquaculture.2017.04.021

Matthäus, B., Piofczyk, T., Katz, H., & Pudel, F. (2019). Renewable resources from insects: exploitation, properties, and refining of fat obtained by cold-pressing from *Hermetia illucens* (Black Soldier Fly) larvae. *European Journal of Lipid Science and Technology*, *121*(7), 1800376. http://doi.org/10.1002/ejlt.201800376

Menconi, A., Pumford, N. R., Morgan, M. J., Bielke, L. R., Kallapura, G., Latorre, J. D., Wolfenden, A. D., Hernandez-Velasco, X., Hargis, B. M., & Tellez, G. (2014). Effect of chitosan on *Salmonella* Typhimurium in broiler chickens. *Foodborne Pathogens and Disease*, *11*(2), 165-169. https://doi.org/10.1089/fpd.2013.1628

Mo, W. Y., Man, Y. B., & Wong, M. H. (2018). Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge. *Science of The Total Environment*, *613*, 635-643. https://doi.org/10.1016/j.scitotenv.2017.08.321

Muin, H., Taufek, N., Kamarudin, M., & Razak, S. (2017). Growth performance, feed utilization and body composition of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed with different levels of black soldier fly, *Hermetia illucens* (Linnaeus, 1758) maggot meal diet. *Iranian Journal of Fisheries Sciences*, *16*, 567-577. https://doi.org/10.1111/anu.12573

Nordentoft, S., Fischer, C., Bjerrum, L., Heckmann, L., & Hald, B. (2017). Reduction of *Escherichia coli*, *Salmonella Enteritidis* and *Campylobacter jejuni* in poultry manure by rearing of Musca domestica fly larvae. *Journal of Insects as Food and Feed*, *3*, 145-153. https://doi.org/10.3920/JIFF2016.0058

Obiero, K., Meulenbroek, P., Drexler, S., Dagne, A., Akoll, P., Odong, R., Kaunda-Arara, B., & Waidbacher, H. (2019). The contribution of fish to food and nutrition security in Eastern Africa: Emerging trends and future outlooks. *Sustainability*, *11*, 1636. https://doi.org/10.3390/su11061636

Oteri, M., Di Rosa, A. R., Lo Presti, V., Giarratana, F., Toscano, G., & Chiofalo, B. (2021). Black soldier fly larvae meal as alternative to fish meal for aquaculture feed. *Sustainability*, *13*, 5447. https://doi.org/10.3390/su13105447

Park, S. I., & Yoe, S. M. (2017). A novel cecropin-like peptide from black soldier fly, *Hermetia illucens*: Isolation, structural and functional characterization. *Entomological Research*, *47*, 115-124. https://doi.org/10.1111/1748-5967.12226

Park, S. I., Chang, B. S., & Yoe, S. M. (2014). Detection of antimicrobial substances from larvae of the black soldier fly, *H ermetia illucens* (Diptera Stratiomyidae). *Entomological Research*, *44*, 58-64. https://doi.org/10.1111/1748-5967.12050

Park, S. I., Kim, J. W., & Yoe, S. M. (2015). Purification and characterization of a novel antibacterial peptide from black soldier fly (*Hermetia illucens*) larvae. *Developmental & Comparative Immunology*, *52*, 98-106. https://doi.org/10.1016/j.dci.2015.04.018

Pauly, D., & Zeller, D. (2017). Comments on FAOs state of world fisheries and aquaculture (SOFIA 2016). *Marine Policy*, *77*, 176-181. https://doi.org/10.1016/j.marpol.2017.01.006

Perednia, D. A., Anderson, J., & Rice, A. (2017). A comparison of the greenhouse gas production of black soldier fly larvae versus aerobic microbial decomposition of an organic feed material. *Research and Reviews: Journal of Ecology and Environmental Sciences*, *5*, 10-16. https://doi.org/10.1016/j.jclepro.2020.122488.

Provost, B., Jouan, V., Hilliou, F., Delobel, P., Bernardo, P., Ravallec, M., Cousserans, F., Wajnberg, E., Darboux, I., Fournier, P., Strand, M. R., & Volkoff, A. -N. (2011). Lepidopteran transcriptome analysis following infection by phylogenetically unrelated polydnaviruses highlights differential and common responses. *Insect Biochemistry and Molecular Biology*, *41*(8), 582-591. http://doi.org/10.1016/j.ibmb.2011.03.010

Rahmi, F. A., Yamin, M., & Sasaerila, Y. (2020). Effect of different organic wastes on the growth of black soldier fly (*Hermetia illucens*) larvae. *Proceedings of the International Conference and the 10th Congress of the Entomological Society of Indonesia (ICCESI 2019)*, Indonesia, pp. 113-116. https://doi.org/10.2991/absr.k.200513.019

Raksasat, R., Lim, J. W., Kiatkittipong, W., Kiatkittipong, K., Ho, Y. C., Lam, M. K., Font-Palma, C., Zaid, H. F. M., & Cheng, C. K. (2020). A review of organic waste enrichment for inducing palatability of black soldier fly larvae: Wastes to valuable resources. *Environmental Pollution*, *267*, 115488. https://doi.org/10.1016/j.envpol.2020.115488

Ravi, H. K., Guidou, C., Costil, J., Trespeuch, C., Chemat, F., & Vian, M. A. (2021). Novel insights on the sustainable wet mode fractionation of black soldier fly larvae (*Hermetia illucens*) into lipids, proteins and chitin. *Processes*, *9*(11), 1888. https://doi.org/10.3390/pr9111888

Ravi, H. K., Vian, M. A., Tao, Y., Degrou, A., Costil, J., Trespeuch, C., & Chemat, F. (2019). Alternative solvents for lipid extraction and their effect on protein quality in black soldier fly (*Hermetia illucens*) larvae. *Journal of Cleaner Production*, *238*, 117861. https://doi.org/10.1016.j.jclepro.2019.117861

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M.T., Biasato, I., & Biasibetti, E. (2017). Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *Journal of Animal Science and Biotechnology*, *8*, 1-13. https://doi.org/10.1186/s40104-017-0191-3

Sakadevan, K., & Nguyen, M. L. (2017). Livestock production and its impact on nutrient pollution and greenhouse gas emissions. *Advances in Agronomy*, *141*, 147-184. https://doi.org/10.1016/bs.agron.2016.10.002

Salomone, R., Saija, G., Mondello, G., Giannetto, A., Fasulo, S., & Savastano, D. (2017). Environmental impact of food waste bioconversion by insects: application of life cycle assessment to process using *Hermetia illucens*. *Journal of Cleaner Production*, *140*, 890-905. https://doi.org/10.1016/j.jclepro.2016.06.154

Secci, G., Mancini, S., Iaconisi, V., Gasco, L., Basto, A., & Parisi, G. (2019). Can the inclusion of black soldier fly in diet affect the flesh quality/nutritional traits of rainbow trout (*Oncorhynchus mykiss*) after freezing and cooking? *International Journal of Food Sciences and Nutrition*, *70*, 161-171. https://doi.org/10.1080/09637486.2018.1489529

Shin, H. S., & Park, S. I. (2019). Novel attacin from *Hermetia illucens*: cDNA cloning, characterization, and antibacterial properties. *Preparative Biochemistry and Biotechnology*, *49*, 279-285. https://doi.org/10.1080/10826068.2018.1541807

Soetemans, L., Uyttebroek, M., D'Hondt, E., & Bastiaens, L. (2019). Use of organic acids to improve fractionation of the black soldier fly larvae juice into lipid-and protein-enriched fractions. *European Food Research and Technology*, *245*(10), 2257-2267. https://doi.org/10.1007/s00217-019-03328-7

Springmann, M., Clark, M., Mason-D'Croz, D., Wiebe, K., Bodirsky, W., Vermeulen, S. J., Herrero, M., & Carlson, K. M. (2018). Options for keeping the food system within environmental limits. *Nature*, *562*, 519-525. https://doi.org/10.1038/s41586-018-0594-0

Stadtlander, T., Stamer, A., Buser, A., Wohlfahrt, J., Leiber, F., & Sandrock, C. (2017). *Hermetia illucens* meal as fish meal replacement for rainbow trout on farm. *Journal of Insects as Food and Feed*, *3*, 165-175. https://doi.org/10.3920/JIFF2016.0056

Su, C. H., Nguyen, H. C., Bui, T. L., & Huang, D. L. (2019). Enzyme-assisted extraction of insect fat for biodiesel production. *Journal of Cleaner Production*, *223*, 436-444. https://doi.org/10.1016/j.jclepro.2019.03.150

Teigiserova, D. A., Hamelin, L., & Thomsen, M. (2020). Towards transparent valorization of food surplus, waste and loss: Clarifying definitions, food waste hierarchy, and role in the circular economy. *Science of The Total Environment*, *706*, 136033. https://doi.org/10.1016/j.scitotenv.2019.136033

Tippayadara, N., Dawood, M. A., Krutmuang, P., Hoseinifar, S. H., Doan, H. V., & Paolucci, M. (2021). Replacement of fish meal by black soldier fly (*Hermetia illucens*) larvae meal: effects on growth, haematology, and skin mucus immunity of Nile tilapia, *Oreochromis niloticus*. *Animals*, *11*, 193. https://doi.org/10.3390/ani11010193

Ushakova, N., Dontsov, A., Sakina, N., Brodsky, E., Ratnikova, I., Gavrilova, N., Bastrakov, A., Kozlova, A., & Nekrasov, R. (2017). Melanin properties at the different stages towards life cycle of the fly *Hermetia illucens*. *Ukrainian Journal of Ecology*, *7*(4), 424-431. https://doi.org/10.15421/2017_137

Verstraete, F. (2013). Risk management of undesirable substances in feed following updated risk assessments. *Toxicology and Applied Pharmacology*, *270*, 230-247. https://doi.org/10.1016/j.taap.2010.09.015

Vongvichith, B., Morioka, S., Sugita, T., Phousavanh, N., Phetsanghanh, N., Chanthasone, P., Pommachan, P., & Nakamura, S. (2020). Evaluation of the efficacy of aquaculture feeds for the climbing perch *Anabas testudineus*: Replacement of fishmeal by black soldier fly *Hermetia illucens* prepupae. *Fisheries Science*, *86*, 145-151. https://doi.org/10.1007/s12562-019-01381-5

Wang, G., Peng, K., Hu, J., Yi, C., Chen, X., Wu, H., & Huang, Y. (2019). Evaluation of defatted black soldier fly (*Hermetia illucens* L.) larvae meal as an alternative protein ingredient for juvenile Japanese seabass (*Lateolabrax japonicus*) diets. *Aquaculture*, *507*, 144-154. https://doi.org/10.1016/j.aquaculture.2019.04.023

Wiseman, S. A., Dötsch-Klerk, M., Neufingerl, N., & de Oliveira Martins, F. (2019). Future food: Sustainable diets for healthy people and a healthy planet. *International Journal of Nutrology*, *12*, 023-028. https://doi.org/10.1055/s-0039-1695714

Xiao, X., Jin, P., Zheng, L., Cai, M., Yu, Z., Yu, J., & Zhang, J. (2018). Effects of black soldier fly (*Hermetia illucens*) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture Research*, *49*, 1569-1577. https://doi.org/10.1111/are.13611

Xu, J., Luo, X., Fang, G., Zhan, S., Wu, J., Wang, D., & Huang, Y. (2020). Transgenic expression of antimicrobial peptides from black soldier fly enhance resistance against entomopathogenic bacteria in the silkworm, Bombyx mori. *Insect Biochemistry and Molecular Biology*, *127*, 103487. https://doi.org/10.1016/j.ibmb.2020.103487

Zarantoniello, M., Bruni, L., Randazzo, B., Vargas, A., Gioacchini, G., Truzzi, C., Annibaldi, A., Riolo, P., Parisi, G., & Cardinaletti, G. (2018). Partial dietary inclusion of black soldier fly full-fat prepupae in zebrafish feed: biometric, histological, biochemical, and molecular implications. *Zebrafish*, *15*, 519-532. https://doi.org/10.3390/ani11030720

Zarantoniello, M., Zimbelli, A., Randazzo, B., Compagni, M. D., Truzzi, Osimani, A., & Milanović, V. (2020). Black soldier fly reared on roasted coffee by-product and *Schizochytrium* sp. as a sustainable terrestrial ingredient for aquafeeds production. *Aquaculture*, *518*, 734659. https://doi.org/10.1016/j.aquaculture.2019.734659

Zhou, J., Liu, S., Ji, H., & Yu, H. (2018). Effect of replacing dietary fish meal with black soldier fly larvae meal on growth and fatty acid composition of Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Nutrition*, *24*, 424-433. https://doi.org/10.1111/anu.12574

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RESEARCH ARTICLE

An overview on the readiness level of the Turkish maritime industry for decarbonization in shipping

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ABSTRACT

Decarbonization is under spotlights for shipping as with many other transportation units. However, the readiness and awareness of the Turkish maritime industry is a common uncertainty. To reveal and show the current progress of the industry, a survey has been carried out. Participants from different companies with different education levels and experiences have been joined and answered the questionnaire which aims to clarify the past, present, and future of the maritime industry. The results of the survey show that the Turkish maritime industry is not fully ready at the company level, however, they perform better at the individual level. Furthermore, the industry may require additional regulation and technical support from maritime stakeholders such as chambers, related government departments, and non-governmental organizations.

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Introduction

Maritime transportation has the major share of 90% of the worldwide trade (Anonymous, 2022a), 90% of the outer freight transportation, and 40% of the inner freight transportation (Fan et al., 2018). According to United Nations Conference on Trade and Development (UNCTAD) (2020), maritime transportation was done by 98.140 commercial ships of 100 gross and above in January 2020 which equals 2,06 billion deadweight tons. Maritime transportation constituted 12% of worldwide transportation energy need (USEIA, 2016) and consumed 300 million tons of fossil fuels which were 72% of residual fuels (heavy fuel oil), 26% distillate fuels (marine diesel oil, marine gas oil), and 2% of liquefied natural gas (LNG) in 2015 (IMO, 2015).

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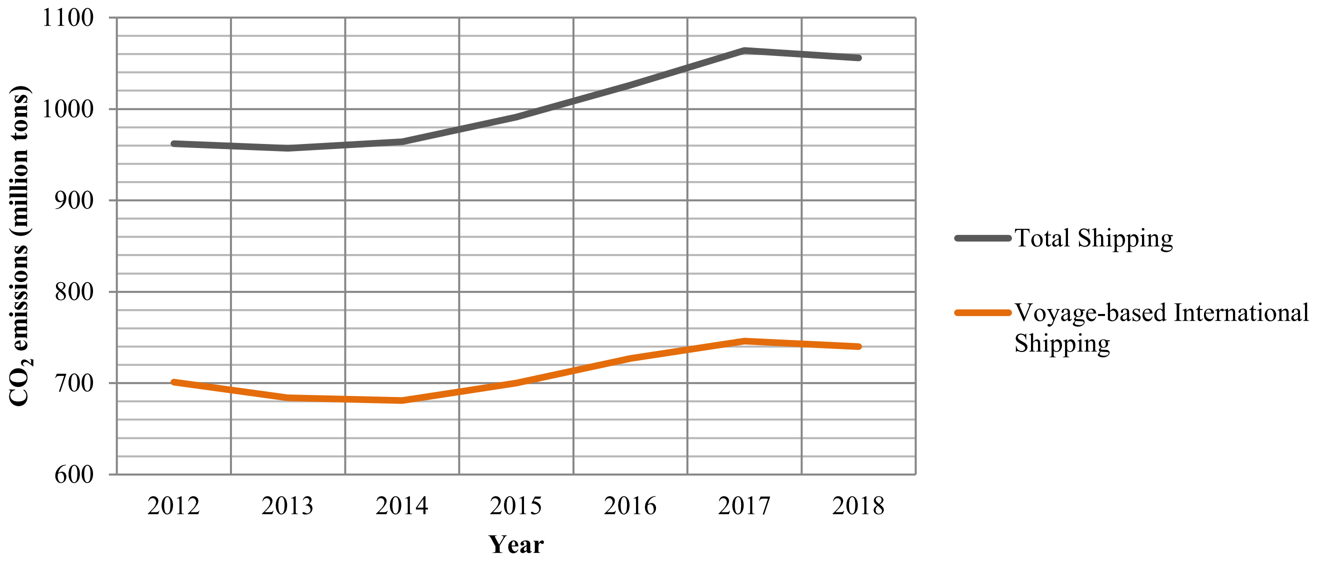

Figure 1. Maritime transportation CO_2 emissions between 2012 and 2018 (data retrieved from IMO, 2020)

Despite maritime transportation being the most efficient way of transportation and emitting less CO_2 per ton-km than other types of transport (Balcombe et al., 2019), it has a considerable contribution to worldwide environmental impact (Lister et al., 2015). A study states that the amount of greenhouse gas (GHG) emissions of international maritime transportation is comparable to the GHG emissions of Germany (Olmer et al., 2017). The maritime transportation GHG emissions, CO_2, methane (CH_4), and nitrous oxide (N_2O), rise from 977 million tons to 1,076 million tons and CO_2 emissions increased from 962 million tons to 1,056 million tons from 2012 to 2018 (IMO, 2020). It can be seen that CO_2 emissions are the major GHG emissions from maritime transportation. Figure 1 shows voyage-based international shipping and total shipping CO_2 emissions between 2012 and 2018.

Maritime transportation CO_2 emissions are in increasing even though the efforts of the International Maritime Organization (IMO), which is an agency of the United Nations that develops regulations for international maritime transportation. It is observed that the increase rate of voyage-based international shipping CO_2 emissions is lower than the total shipping CO_2 emissions increase rate. The reason is IMO's efforts to control and mitigate CO_2 emissions slows down the increase rate of the voyage-based international shipping, on the other hand, the global fleet number is increased and the total shipping CO_2 emission increase rate is higher. The total CO_2 emissions from maritime transportation are approximately 3.1% of the total worldwide CO_2 emissions, but IMO predicts that the amount of the CO2 emissions will grow between 50% to 250 by 2050 (IMO, 2015) if there will be no mitigation plan or strategy.

IMO started its effective intervention to mitigate CO_2 emissions with the Regulations on Energy Efficiency for Ships. This regulation entered into force on 1 January 2013 by an amendment to MARPOL Annex VI (IMO, 2011). The purpose of the regulation is to control and mitigate CO_2 emissions both from the new building and existing ships. The regulation came with terms, the Energy Efficiency Design Index (EEDI), the Ship Energy Efficiency Management Plan (SEEMP), and the Energy Efficiency Operational Indicator (EEOI). The EEDI is a mandatory design index for the new building ships. It determines the design index limits for specific ship types and directs the use of energy-efficient materials, technologies, or engines. The maximum allowable EEDI limit has been reduced every five years by 10% in general (Dere & Deniz, 2020), but it varies according to specific ship types. The SEEMP is another mandatory term that aims to increase shipboard operation efficiency of ships doing international maritime transportation. The SEEMP comprises operational measures such as ship speed optimization, speed reduction (slow steaming), trip optimization, weather routing, etc. Moreover, the EEOI is a voluntary operational indicator that shows voyage-based CO_2 emissions per transport work of ships (Zincir & Deniz, 2016).

The latest regulation of IMO on CO_2 emissions is IMO Data Collection System (DCS) which is an amendment again to MARPOL Annex VI and is entered into force on 1 March 2018 (Anonymous, 2022b). The regulation aims to record the annual CO_2 emissions from ships larger than 5000 gross tonnage and above. After the emission data is recorded by the shipping

Table 1. Candidate measures of IMO Initial GHG Strategy (IMO, 2018)

Short-term Measures	**Mid-term Measures**	**Long-term Measures**
Enhancement of EEDI and SEEMP	Implementation program for alternative low-carbon and zero-carbon fuels, including an update of national action plans	Development and provision of zero-carbon or fossil-free fuels
Development of technical and operational energy efficiency measures	Operational energy efficiency measures for both new and existing ships	Encourage and facilitate the general adoption of other possible new/innovative emission reduction mechanisms
Establishment of an existing fleet improvement program	Market-based measures for emission reduction	
Speed optimization, speed reduction	Further, continue and enhance technical cooperation and capacity-building activities	
Measures to reduce methane and volatile organic compounds	Development of a feedback mechanism	
Development and update of national action plans		
Continue and enhance technical cooperation and capacity-building activities		
Encourage port developments and activities		
Initiation of research and development activities on marine propulsion, alternative low-carbon, and zero-carbon fuels, and innovative technologies		
Incentives for initiators of new technology development		
Development of lifecycle GHG/carbon intensity guidelines for all types of fuels		
Undertake additional GHG emission studies		

companies, data have to be reported to the flag State after the end of each calendar year. Furthermore, IMO has an Initial Greenhouse Gas Strategy for maritime transportation that aims to diminish GHG emissions by 50% in 2050, compared to 2008 (ICCT, 2018). The Initial Strategy also aims to reduce CO_2 emissions per transport work at least 40% and 70% in 2030 and 2050, respectively, when it is compared to 2008. It is the first action of IMO to help the worldwide goals of the Paris Agreement on climate change (Serra & Fancello, 2020). IMO proposes candidate measures for short-term (2018-2023), mid-term (2023-2030), and long-term (2030-…) in the Initial Strategy (IMO, 2018). Table 1 shows candidate measures for short-, mid-, and long-term.

IMO announced its Initial GHG Strategy, but the route to achieving GHG, especially CO_2 emissions, reduction goal is left to maritime stakeholders and flag states. At the meeting, IMO MEPC 75 on 16-20 November 2020, new technical and operational measures, the Energy Efficiency Existing Ship Index (EEXI) and the Carbon Intensity Indicator (CII), respectively, were adopted (de Kat, 2020) to speed up the decarbonization action of global maritime transportation. The EEXI is a technical measure and is going to enter into force on

1 January 2023. It is similar to the EEDI and shows the energy efficiency level of the ship, but it is going to be applied to existing ships. An attained EEXI will be calculated for each existing ship, and this index has to meet the required maximum EEXI of this type of ship. On the other hand, the CII is a new operational indicator that will be applied after 2025. The annual operational CII will be collected from shipping companies as part of the IMO DCS, and an operational rating from A to E (five-point scale) will be given to each ship (Psaraftis & Kontovas, 2021). The CII does not have any strict enforcement, but well-known cargo shippers can prefer higher rated-ships to transport their cargoes.

Various ways or combinations of different ways provide CO_2 emission reduction. Shipboard efficiency improvement actions on compressed air systems (Dere & Deniz, 2019a) or cooling water systems (Dere & Deniz, 2019b) can decrease CO_2 emissions to some extent. Balcombe et al. (2019) state that LNG is the main actor in alternative fuels. Biofuels, hydrogen, nuclear power, electric propulsion, and carbon capture and storage system are some of the routes for higher decarbonization. The study of Yalcin & Suner (2020) shows that using hydrogen reduces carbon emissions and related health risks (Suner & Yalcin, 2017; Yalcin & Suner, 2020). Using methanol as an alternative fuel with an advanced combustion concept (Zincir et al., 2019) and fuel cell application on a ship (Inal & Deniz, 2020; Inal & Deniz, 2021) can be other decarbonization methods in maritime transportation. To achieve effective decarbonization, multiple measures should be applied and stronger policy is required. A study was made on a systematic assessment of the technical feasibility of decarbonized maritime transport by 2035 (Halim et al., 2018). According to the study, the governments should involve in the decarbonization action and put some policies or regulations including zero-carbon operations, more stringent energy efficiency targets, a speed limit, and a low-carbon fuel standard. Moreover, ports' infrastructure should be improved by shore power facilities for cold ironing, battery charging stations, and alternative fuel bunkering facilities.

The IMO Initial GHG Strategy is a complicated process and there are various organizational, economic, technical, and political challenges and barriers (Serra & Fancello, 2020). The Strategy should be discussed more in detail from the perspective of both policymakers and ship owners/operators. The flag states which agreed on the Strategy should prepare their roadmap to remove the barriers for decarbonization of maritime transportation and contribute to the global decarbonization action by organizing shipowners and ship operators.

There are some studies in the literature that are based on interviews with the industry to see the progress of decarbonization in various sectors. Sovacool et al. (2018) have a study on national and regional transport challenges of Nordic countries about climate policy priorities. The study had 227 expert interviews from Denmark, Finland, Iceland, Norway, and Sweden. The experts are the stakeholders of transport technology, policy, and practice. The results of the study showed that fossil fuel intensity (42%) was the highest challenge, long travel distances (17%), public transport infrastructure (16%), congestion (15%), population density (10%), and electrification of transport (10%) follow it. A qualitative interview study was made with Greek shipowners about the decarbonization of maritime transportation (Koustoumpardis, 2019). The shipowners indicate that the LNG as an alternative fuel and electric propulsion are the main routes for the decarbonization of maritime transportation. Furthermore, an appropriate legislative framework must be established for successful decarbonization. Shell & Deloitte (2020) did a market survey study to understand the maritime industry trends. According to their study, more than 90% of maritime industry stakeholders count decarbonization as their business strategy. Another sector-based study was conducted in Italy (Sofia et al., 2020). The study focused on the decarbonization of various sectors including energy, transport, and household in 2030. According to the study, electrification is the road map for the decarbonization of maritime transportation in 2030. The world's largest container shipping company, Maersk, has declared their objective to be zero-carbon by 2050 (A.P. Moller-Maersk, 2018).

Turkey is one of the important flag states with its 1528 vessels (449 national flagged-vessel, 1079 foreign flagged-vessel) above 1000 gross tons and above (UNCTAD, 2020). Nevertheless, when the literature search is done, there are not many studies on the decarbonization of Turkish maritime transportation. The status of Turkish maritime transportation and opinions of Turkish shipowners and ship operators on the decarbonization of maritime transportation is unknown. This study aims to fill the literature gap by conducting a survey study. A questionnaire about the decarbonization of maritime transportation is formed and it is sent to Turkish shipowners and ship operators. The answers to the questionnaires are analyzed and the status and opinions of the Turkish maritime sector are discussed.

Table 2. Participants' profile

	Qualification	*Percentage*
Education Level	Undergraduate	60,1
	Master	32,1
	Doctorate	7,8
Department	Management	5,3
	Operation	21,1
	Technical	47,9
	Personal	15,3
	Other	10,5
Company Experience	1-3 years	26,8
	4-6 years	31,6
	7-9 years	26,3
	10+ years	15,3
Onboard Ship Experience	1-3 years	37,4
	4-6 years	31,6
	7-9 years	15,3
	10+ years	15,8

Table 3. Question frames were used in the survey

Notation	*Topic Description*
T1	Assess the knowledge level about maritime decarbonization strategies.
T2	Assess the company culture about decarbonization.
T3	Assess the knowledge about EEDI, SEEMP, and EEOI.
T4	Assess the knowledge about EEXI, and CII.
T5	Assess the usage ability of DCS and MRV.
T6	Assess the seaman's knowledge about decarbonization.
T7	Assess the company procedures and improvements on the decarbonization.
T8	Assess the operational energy efficiency applications.
T9	Assess the point of view market-based measures approaches such as taxes, levies.
T10	Assess the point of view on renewable energy sources for decarbonization.
T11	Assess the point of view on hybrid and electrical propulsion.
T12	Assess the point of view on capital and operational expenditures of new technologies.
T13	Assess the point of view on the maritime education curriculum.
T14	Assess the point of view on the governmental approaches.
T15	Assess the point of view on the class societies.
T16	Assess the point of view on the professional chambers and NGOs.

Note: Abbreviations: DCS: Data Collection System; EEDI: Energy Efficiency Design Index; SEEMP: Ship Energy Efficiency Management Plan; EEOI: Energy Efficiency Operational Index; EEXI: Energy Efficiency Existing Ship Index; CII: Carbon Intensity Index; MRV: Monitoring, Reporting, and Verification; NGO: Non-governmental Organizations.

Methodology

To understand the point of view and current status of the maritime industry, a questionnaire which is formed by 29 questions has been prepared. The survey contains different questions types to match the research data. Four questions types; multiple-choice, numeric open-ended, Likert scale, and ranking scales have been used. The descriptive information of the participants and their percentages are given in Table 2. The questionnaire answers are entered into SPSS Statistics Version 25.0 to analyze the data. According to gathered data from the participants, four main categories have been formed; education level, department, company experience, and onboard ship experience. The categories will be used to elaborate and understand the bias among different responses for the same questions.

As can be seen in Table 2, the four main categories have different subtitles. The education level of the participants is divided into three undergraduate levels, a master level, and a doctorate level. The graduate-level participants are the majority but slightly in front of the master level. However, participants with a doctorate are the minority as expected. Secondly, participants are also categorized according to their departments. Companies may have different departments however, the major four -management, operation, technical, and personal- are the same for all. Therefore, besides these four departments, another is also added and 5 subtitles are formed for the department category. The majority of the participants are from the technical department of the companies. Thirdly and fourthly, company and onboard ship experiences are selected as categories. Both are divided into same-year scales; 1-3, 4-6, 7-9, and 10+ years. The majority of the participants are in 1-3 years.

The questionnaire has been formed by 29 questions from different perspectives. Therefore, they are clustered under 16 topics and given as question frames in Table 3.

Results and Discussion

The responses were given in five scales where 1 is very weak and 5 is very good. Table 4 gives the evaluation scales for each question, Table 5, Table 6, Table 7 and Table 8 give the mean of the responses respecting education level, department, company experiences, and onboard ship experiences of the participants, respectively.

Table 4. Evaluation scales for the questions

Evaluation Scale	Indication
1	Very Weak
2	Weak
3	Moderate
4	Good
5	Very Good

Table 5 shows the mean value of the answers according to the education level of the participants and the total average value of the answers. The values of T1 – knowledge level of maritime decarbonization strategies, T3 – knowledge about EEDI, SEEMP, and EEOI, and T14 – governmental approaches indicate no relation to the education level. Because the mean values under three education levels do not show a meaningful trend. When T2 results are checked, it is observed that the participants with a higher education level think that the company culture on decarbonization action does not adequate. The doctorate-level participants have a lower mean value of 2,33 than the total average value of 3,16. These participants see company culture on decarbonization as weak. T4 – knowledge of EEXI, CII results show that the participants with a higher education level think that the company that they work do not aware of new decarbonization regulations such as EEXI or CII. The participants with doctorate-level think that the company has a very weak awareness of new decarbonization regulations by the mean value of 1,67. T5 – usage ability of DCS and MRV

Table 5. Results of the topics according to the education level of participants

Topic No	Undergraduate	Master	Doctorate	Average
T1	3	4.13	3	3.47
T2	3.38	3.25	2.33	3.16
T3	4	4	4	4
T4	3.13	3.38	1.67	3
T5	3.63	3.13	3.34	3.37
T6	2.5	2.75	2	2.53
T7	3.38	3	2.67	3.1
T8	2.63	3.38	3.34	3.05
T9	4.5	4	3.67	4.16
T10	4.5	3.75	3.67	4.05
T11	4.5	4.13	4.67	4.37
T12	3.88	4.25	4.33	4.11
T13	1.88	1.5	2.67	1.84
T14	2	1.75	2	1.89
T15	2.5	2.37	2.67	2.47
T16	1.75	1.5	2	1.68

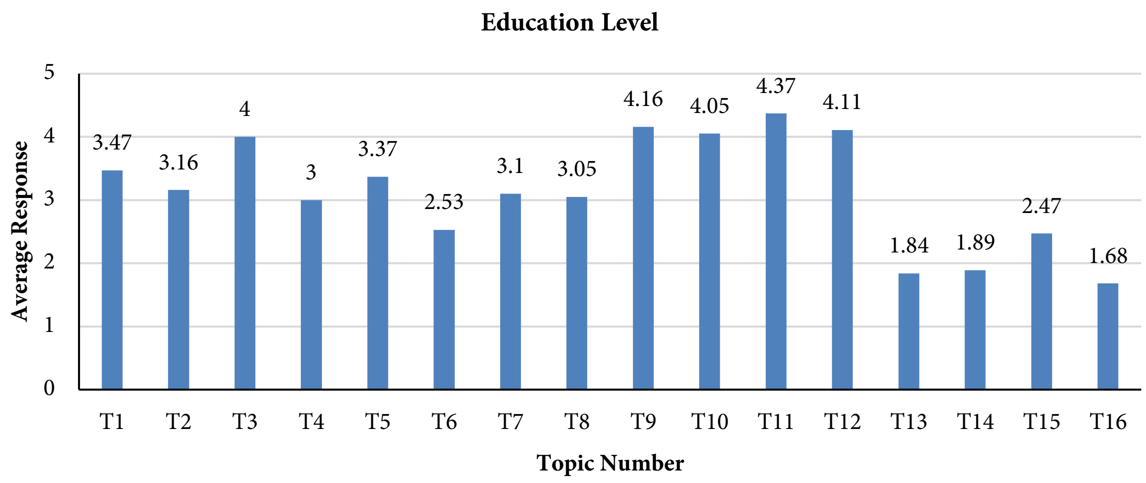

Figure 2. Average response results of the topics according to the education level of the participants

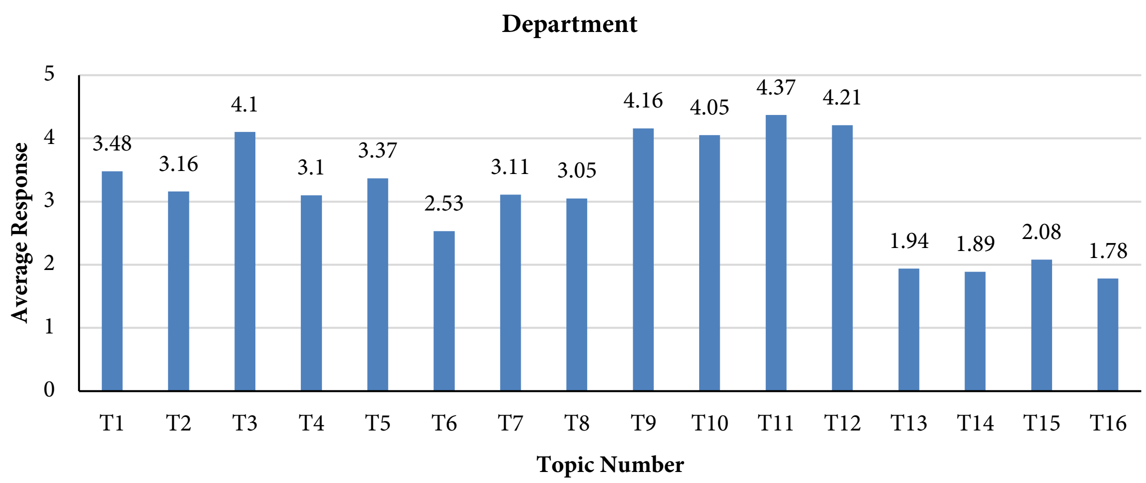

Figure 3. Average response results of the topics according to the department of the participants

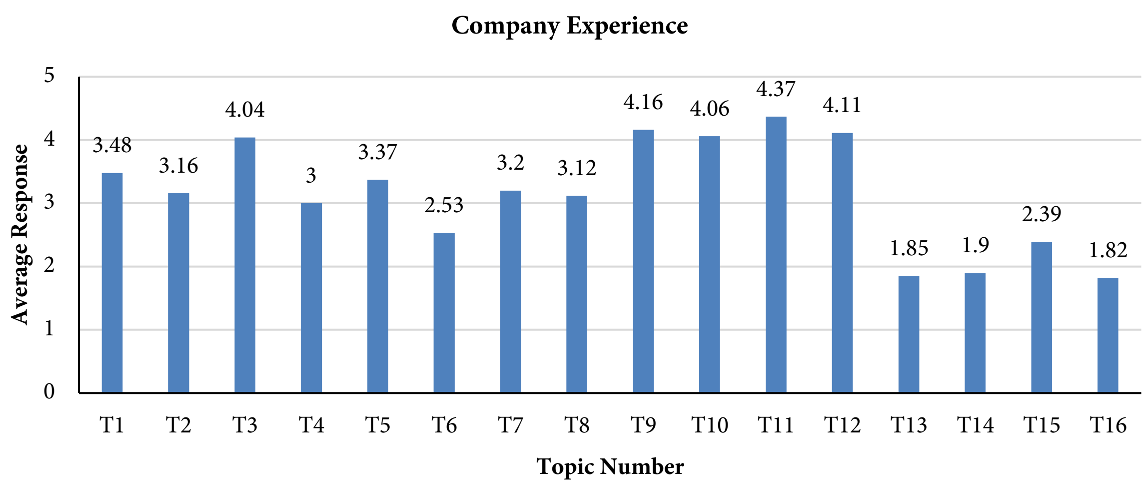

Figure 4. Average response results of the topics according to the company experience of the participants

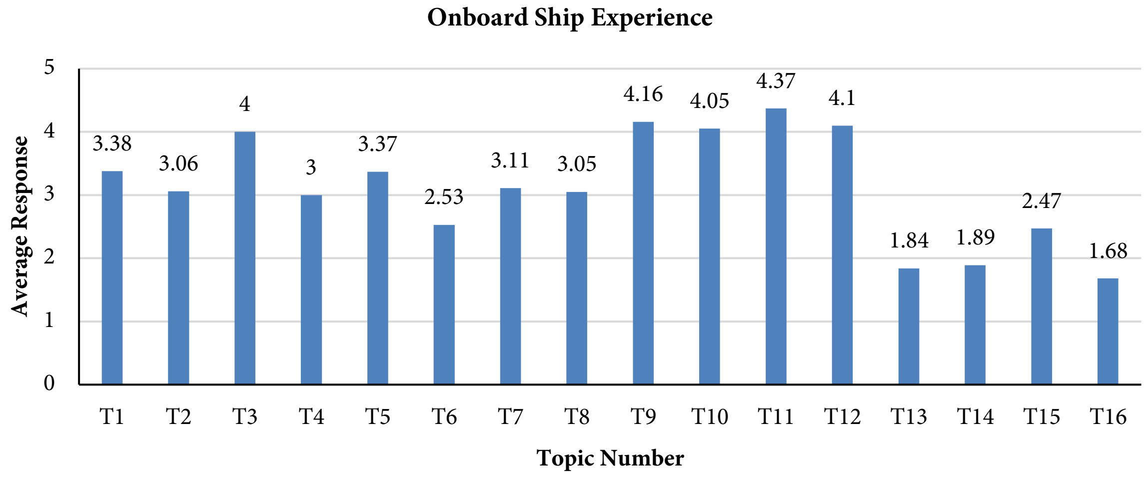

Figure 5. Average response results of the topics according to the onboard ship experience of the participants

results slightly change with the education level of participants. The participants with doctorate-level think that the company has a very weak awareness of new decarbonization regulations by the mean value of 1,67. T5 – usage ability of DCS and MRV results slightly change with the education level of participants. The participants with a higher education level think that the DCS and MRV are moderately applied, but with a lower mean value. With a higher education level, the participants think that the crew knowledge on decarbonization (T6) action is lower. The higher educated participants also answer with lower values at company procedures and improvements on decarbonization (T7) topic, which means the higher educated participants do not think that the procedures and improvements are enough for the decarbonization action. T8 – operational energy efficiency applications results show that higher educated participants are more satisfied with the applications on ships. On the other hand, when the education level increases the mean value of the answer reduces. Since these participants do not think that market-based measures (MBMs) are an effective solution for the decarbonization of shipping (T9). It is a surprise that the participants with a higher education level give lower value at T10 – renewable energy sources. The master level (3,75) and doctorate level (3,67) participants think renewable energy sources are a moderately effective solution for decarbonization. This can be because these participants have higher knowledge of renewable energy systems, and do not think that they are easy to apply on ships in the Turkish fleet. On the other hand, master and doctorate-level participants think that hybrid and electric propulsion can be a good option for decarbonization (T11). But they also answer that capital and operational expenditures are important factors for the new technologies (T12). The higher mean value with the higher education level can be related to higher knowledge and awareness of the participants on the new technologies with the increasing education level. One of the important topics, T13 – the maritime education curriculum, the result shows that in general nobody thinks maritime education is adequate for the decarbonization action in Turkey. They give points between 1,5 and 2,67 which corresponds to very weak and between weak and moderate, respectively. The participants with doctorate-level give the highest points with 2,67. It can be because they got education from undergraduate to doctorate level and they think that they have more enough knowledge on decarbonization. The higher educated participants think that class societies (T15) and chambers and NGOs (T16) in Turkey have knowledge and awareness on decarbonization action from weak to moderate (2.67) and weak (2), respectively.

Table 6 shows the mean value of the answers according to the department of each participant and the total average value of the answers. The values of T1 – knowledge level of maritime decarbonization strategies, and T3 – knowledge about EEDI, SEEMP, and EEOI are directly showing that the participants from the management and technical departments of the companies are much more aware of the way of decarbonization in the global maritime industry. T4 – knowledge of EEXI, CII, and T5 – DCS and MRV usage results show that the participants from management and technical departments of the company consider that their knowledge is fairly well and relatively enough for the industry. When T2 results are checked, it is observed that the participants from operational and personal departments think that the company culture on decarbonization action does not adequate. As expected, the participants from the management and personal department think that the crew knowledge on decarbonization (T6) action

Table 6. Results of the topics according to the department of participants

Topic No	Management	Operational	Technical	Personal	Other	Average
T1	4.7	2.5	4.21	3.6	2.5	3.48
T2	4.78	2.25	3.46	2	3	3.16
T3	4.8	3.5	4.45	2.1	3.2	4.1
T4	3.55	2.2	4.1	2	2.1	3.1
T5	3.66	3.25	4.8	2.2	2.8	3.37
T6	4.1	2.25	2.36	4.07	2.5	2.53
T7	4.9	2.25	3.37	2.1	2.9	3.11
T8	4.68	2.75	3.1	2.9	3	3.05
T9	4.9	3.76	4.27	2.1	1.85	4.16
T10	4.8	3.75	4.18	4	4	4.05
T11	4.9	3.75	4.64	3.2	4.1	4.37
T12	4.9	4.2	4.1	2.3	4.7	4.21
T13	3.1	2.5	1.46	4.1	1.2	1.94
T14	2	1.5	1.97	3.6	1.5	1.89
T15	2.8	2.78	2.45	2.2	1.1	2.08
T16	2	1.25	1.91	1.4	1.6	1.78

Table 7. Results of the topics according to the company experience of participants

Topic No	1-3 years	4-6 years	7-9 years	10+ years	Average
T1	3	3.43	3.8	4.9	3.48
T2	3.42	2.83	2.8	4.7	3.16
T3	3	4.17	4.6	4.82	4.04
T4	3.29	2.33	3.2	4	3
T5	3.29	3.67	2.89	5	3.37
T6	2.29	2.33	2.81	4	2.53
T7	3.57	2.5	2.64	4.9	3.2
T8	3.14	2.53	3.21	4.8	3.12
T9	4.14	4	4.2	5	4.16
T10	3.72	4.17	4.4	4	4.06
T11	4.43	4.33	4.2	4.7	4.37
T12	4.43	4	3.6	5	4.11
T13	1.57	2.04	1.84	3.08	1.85
T14	1.43	2	2.41	2.13	1.9
T15	2.57	2.67	1.65	4.23	2.39
T16	2.17	1.72	1.68	2.31	1.82

is high. However, results from other departments are in contrast. The participants from management also answer with higher values at company procedures and improvements on decarbonization (T7) topic and operational energy efficiency applications (T8), which means the management team thinks that the procedures and improvements are enough for the decarbonization action. Almost all participants think that market-based measures (MBMs) are an effective solution for the decarbonization of shipping (T9) since the average is one of the highest. The participants from whole departments think renewable energy sources (T10) and hybrid and electric propulsion (T11) can be good options and effective solutions for decarbonization. Hybrid systems show slightly better performance compared to renewable energy, this can be as same as table 5, because these participants have higher knowledge of renewable energy systems, and do not think that they are easy to apply on ships in the Turkish fleet. When T13 – the maritime education curriculum, T14 – governmental approaches, T15 – class societies, and lastly T16 – NGOs and chambers are under the scope, the participants give the lowest points in general. The result shows that nobody thinks neither maritime education is adequate for the decarbonization action nor chambers and NGOs (T16) in Turkey have knowledge and awareness on decarbonization.

Table 8. Results of the topics according to onboard ship experience of participants

Topic No	1-3 years	4-6 years	7-9 years	10+ years	Average
T1	3.67	2.67	4.61	4	3.38
T2	2.89	3	4.69	3.67	3.06
T3	3.67	3.83	5	5	4
T4	2.78	2.33	4.9	4.33	3
T5	2.56	3.83	5	4.33	3.37
T6	2.33	2.33	4.5	2.67	2.53
T7	2.89	2.83	5	3.67	3.11
T8	3	2.67	4.6	3.33	3.05
T9	3.89	4.17	5	4.67	4.16
T10	3.89	4.33	2.3	4.67	4.05
T11	4.22	4.33	4.8	4.67	4.37
T12	4.2	4.33	4.1	4	4.1
T13	1.89	1.67	2	2	1.84
T14	2	1.8	2	1.67	1.89
T15	2.67	2.17	2	2.67	2.47
T16	1.89	1.5	1	1.66	1.68

Table 7 shows the mean value of the answers according to the company experience for each participant and the total average value of the answers. In general scope, it can be said that the more experienced participants are more aware of the decarbonization in shipping. The values of T1 are drawing a meaningful trend line in coherence with experience. The same point of view is valid also for other topics. From T1 to T12, the knowledge of decarbonization, usage of alternative energy systems, crew awareness, and related regulations and equipment ability is increasing with company experience. This can be clearly observed from the answers of the 10 years plus experienced participants. The mean value of the experienced participants is higher than 4/5, therefore they believe that the knowledge, awareness, technological maturity, and base are enough at the personal and company level. However, the last four topics, T13, T14, T15, and T16 show the worse performance according to participants. Education curriculum, alternative supports, and informative letters from chambers, NGOs, or governmental branches seem like the weakest points in front of the Turkish maritime industry on the decarbonization roadmap.

Table 8 shows the mean value of the answers according to the onboard ship experience of each participant and the total average value of the answers. In general, while the sea experience increases, the general knowledge on the requirements of the decarbonization for the industry increases. T3, T9, T10, T11, and T12 have received greater points and showed better performance according to almost from all the participants from every scale of experience. This means, the participants believe that although capital and operational expenditures are high, renewable energy and hybrid propulsion systems are important on the way of the decarbonization. Moreover, the results are showing that the participants have more information on the current documentation rather than the incoming ones. This is the perfect reflection of difference between T3 and T4. On the other hand, T13, T14, T15, and T16 are rated as the weakest points for the industry in front of the decarbonization. All participants consider that the maritime education and also governmental, societies and NGOs contribution to the industry are not enough for this new era.

Conclusion

In this paper, a survey study has been carried out to show the current status of the Turkish maritime industry on decarbonization action. The questionnaire with 29 questions was prepared and sent to the participants from different companies with different departments, education levels, and experiences. By this questionnaire, the readiness and awareness level of the Turkish maritime industry were evaluated. The main findings of the study are:

- According to the analysis of the participant answers by the education level, higher educated participants think that company culture on decarbonization, knowledge on upcoming decarbonization regulations, application of DCS and MRV, crew knowledge on decarbonization action, company procedures on decarbonization, and MBMs to achieve decarbonization are not adequate. Moreover, they think that the knowledge of the class societies, NGOs, and chambers in Turkey do not have sufficient knowledge to assist companies. All participants, regardless of their education, think that the maritime curriculum is weak.

- Depending on the departments, the analysis shows that management and technical departments are much more aware of the decarbonization action. The knowledge of DCS and MRV usage and upcoming regulations of EEXI and CII is fairly well for these departments. On the other hand, operational and personal departments think that the company culture on decarbonization does not adequate. The crew knowledge on decarbonization is high according to the management and personal departments. All departments think that renewable energy sources and hybrid and electric propulsion can be good options for decarbonization. On the contrary, maritime education curriculum, governmental approaches, knowledge of the class societies, NGOs, and chambers get the lowest points in general.
- It is observed that the participants with more company experience are more aware of the decarbonization in shipping. The knowledge of decarbonization, usage of alternative energy systems and options, crew awareness, and related regulations increase with the company experience. The weakest points are the maritime education curriculum, informative letters from chambers, NGOs, or governmental branches.
- Another observation is while the sea experience increases the knowledge on the decarbonization action increases. The participants believe that renewable energy and hybrid and electric propulsion systems are important for decarbonization, despite their capital and operational expenditures. Generally, the participants have more information on the current regulations rather than the incoming ones. Another common point is all participants think that maritime education and the contributions of the class societies, NGOs, chambers, and governmental branches are not sufficient for decarbonization at Turkish maritime industry.

The main conclusions of this survey study are the maritime education curriculum has to be updated by considering decarbonization actions and maritime stakeholders such as class societies, NGOs, chambers, governmental branches have to give adequate informative letters, bulletins, seminars, etc. to improve the knowledge and awareness of the Turkish maritime industry on the decarbonization of shipping. In the future studies, periodical surveys can be performed to see the improvement of the industry and to determine the readiness level of the new technologies. This study shows a preliminary analysis as an overview of the industry.

Compliance With Ethical Standards

Authors' Contributions

Author BZ designed and wrote the study, OBI performed and managed statistical analyses and wrote the study, ÇD prepared the questionnaire and did the survey study, CD did supervision on the study. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

A.P. Moller-Maersk. (2018). Sustainability Report. A.P. Moller-Maersk: Copenhagen, Denmark.

Anonymous. (2022a). International Chamber of Shipping. Shipping and World Trade. Retrieved from: http://www.marisec.org/shippingfacts/worldtrade/index/php

Anonymous. (2022b). International Maritime Organization (IMO). IMO Data Collection System. Retrieved from: http://www.imo.org/en/ourwork/environment/pollutionprevention/airpollution/pages/data-collection-system.aspx

Balcombe, P., Brierley, J., Lewis, C., Skatvedt, L., Speirs, J., Hawkes, A., & Staffell, I. (2019). How to decarbonise international shipping: options for fuels, technologies and policies. *Energy Conversion and Management*, *182*, 72-88. https://doi.org/10.1016/j.enconman.2018.12.080

de Kat, J. O. (2020). MEPC 75 Outcomes and Industry Implications. American Bureau of Shipping, 8 December 2020, Copenhagen.

Dere, C., & Deniz, C. (2019a). Energy efficiency based operation of compressed air system on ships to reduce fuel consumption and CO_2 emission. *The International Journal of Maritime Engineering*, *161*(Part A2), https://doi.org/10.5750/ijme.v161iA2.1088

Dere, C., & Deniz, C. (2019b). Load optimization of central cooling system pumps of a container ship for the slow steaming conditions to enhance the energy efficiency. *Journal of Cleaner Production*, *222*, 206-217. https://doi.org/10.1016/j.clepro.2019.03.030

Dere, C., & Deniz, C. (2020). Effect analysis on energy efficiency enhancement of controlled cylinder liner temperatures in marine diesel engines with model based approach. *Energy Conversion and Management*, *220*, 113015. https://doi.org/10.1016/j.enconman.2020.113015

Fan, Y. V., Perry, S., Klemes, J. J., & Lee, C. T. (2018). A review on air emissions assessment: transportation. *Journal of Cleaner Production*, *194*, 673-684. https://doi.org/10.1016/j.clepro.2018.05.151

Halim, R. A., Kirstein, L., Merk, O., & Martinez, L. M. (2018). Decarbonization pathways for international maritime transport: a model-based policy impact assessment. *Sustainability*, *10*, 2243. https://doi.org/10.3390/su10072243

Inal, O. B., & Deniz, C. (2021). Emission analysis of LNG fuelled molten carbonate fuel cell system for a chemical tanker ship: a case study. *Marine Science and Technology Bulletin*, *10*(2), 118-133. https://doi.org/10.33714/masteb.827195

Inal, O.B., & Deniz, C. (2020). Assessment of fuel cell types for ships: based on multi-criteria decision analysis. *Journal of Cleaner Production*, *265*, 121734. https://doi.org/10.1016/j.jclepro.2020.121734

International Council on Clean Transportation (ICCT). (2018). The International Maritime Organization's initial greenhouse gas strategy.

International Maritime Organization (IMO). (2011). Resolution MEPC.203(62), Annex 19, Adopted on 15 July 2011. Amendments to the annex of the protocol of 1997 to amend the international convention of pollution from ships, 1973, as modified by the protocol of 1978 relating thereto (inclusion on energy efficiency for ships in MARPOL Annex VI).

International Maritime Organization (IMO). (2015). Third IMO Greenhouse Gas Study 2014.

International Maritime Organization (IMO). (2018). Adoption of the initial IMO strategy on reduction of GHG emissions from ships and existing IMO activity related to reducing GHG emissions in the shipping sector. Note by the International Maritime Organization to the UNFCCC Talanoa Dialogue.

International Maritime Organization (IMO). (2020). Forth IMO Greenhouse Gas Study 2020.

Koustoumpardis, K. (2019). Decarbonisation of Maritime Transport – How does maritime industry lead the way towards decarbonisation? Greek shipowners' perspective. [M.Sc. Thesis. University of Gothenburg].

Lister, J., Poulsen, R. T., & Ponte, S. (2015). Orchestrating transnational environmental governance in maritime shipping. *Global Environmental Change*, *34*, 185-195. https://doi.org/j.gloenvcha.2015.06.011

Olmer, N., Comer, B., Roy, B., Mao, X., & Rutherford, D. (2017). Greenhouse gas emissions from global shipping, 2013-2015. Retrieved from https://theicct.org/wp-content/uploads/2021/06/Global-shipping-GHG-emissions-2013-2015_ICCT-Report_17102017_vF.pdf

Psaraftis, H. N., & Kontovas, C. A. (2021). Decarbonization of maritime transport: is there light at the end of the tunnel? *Sustainability*, *13*, 237. https://doi.org/10.3390/su13010237

Serra, P., & Fancello, G. (2020). Towards the IMO's GHG goals: a critical overview of the perspectives and challenges of the main options for decarbonizing international shipping. *Sustainability*, *12*, 3220. https://doi.org/10.3390/su12083220

Shell & Deloitte. (2020). Decarbonising shipping: All hands on deck: Industry perspectives. Retrieved from https://www.shell.com/promos/energy-and-innovation/decarbonising-shipping-all-hands-on-deck/_jcr_content.stream/1594141914406/b4878c899602611f78d36655ebff06307e49d0f8/decarbonising-shipping-report.pdf

Sofia, D., Gioiella, F., Lotrecchiano, N., & Giuliano, A. (2020). Cost-benefit analysis to support decarbonization scenario for 2030: A case study in Italy. *Energy Policy*, *137*, 111137. https://doi.org/10.1016/j.enpol.2019.111137

Sovacool, B. K., Noel, L., Kester, J., & de Rubens, G. Z. (2018). Reviewing Nordic transport challenges and climate policy priorities: expert perceptions of decarbonisation in Denmark, Finland, Iceland, Norway, Sweden. *Energy*, *165*, 532-542. https://doi.org/10.1016/j.energy.2018.09.110

Suner, M., & Yalcin, E. (2017). Ship emissions and human health relationship: A theoretical and numerical investigation in Asyaport. In Karakoç T., Colpan C., & Şöhret Y. (Eds.), *Advances in Sustainable Aviation*. Springer. https://doi.org/10.1007/978-3-319-67134-5_14

United Nations Conference on Trade and Development (UNCTAD). (2020). Review of Maritime Transport 2020.

US Energy Information Administration (USEIA). (2016). International Energy Outlook 2016.

Yalcin, E., & Suner, M. (2020). The changing role of diesel oil-gasoil-LPG and hydrogen based fuels in human health risk: a numerical investigation in ferry ship operations. *International Journal of Hydrogen Energy*, *45*, 3660-3669. https://doi.org/10.1016/j.ijhydene.2019.02.238

Zincir, B., & Deniz, C. (2016). Investigation of effect of alternative marine fuels on energy efficiency operational indicator (EEOI). *Proceedings of The Second Global Conference on Innovation in Marine Technology and the Future of Maritime Transportation,* Turkey, pp. 713-719.

Zincir, B., Deniz, C., & Tunér, M. (2019). Investigation of environmental, operational and economic performance of methanol partially premixed combustion at slow speed operation of a marine engine. *Journal of Cleaner Production*, *235*, 1006-1019. https://doi.org/10.1016/j.clepro.2019.07.044

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RESEARCH ARTICLE

An evaluation of the effects of human factors on potential ship accidents under pilotage

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ABSTRACT

In recent years, despite the technological advances, and increasing security measures in the maritime industry, it is observed that the effect of the human factor in the marine accidents has not changed. Most of the accidents occur in narrow canals, straits, rivers and entering port areas, resulting in environmental pollution, economical casualties and injury/loss of life. Pilotage is set compulsory in order to maintain safe passage at such confined waters. The purpose of this study is to investigate the effect of critical human factors on the potential ship accidents under pilotage operations. To explore the identified human factors, depth interviews and a questionnaire study were conducted with masters and pilots. The obtained data was analysed using DEMATEL (Decision Making Trial and Evaluation Laboratory) method to identify the most important and influential factors. The DEMATEL method is used to investigate interaction among human factors and to visualize them with help of causal-effect relation diagram. The results show that master experience, pilot experience and crew training are significant factors compared to other human risk factors. As a result of the findings of this research, it is also thought that improving the collaboration and communication between the master and the pilot will be effective in preventing the accidents. Moreover, understanding casual relations among human factors is important to prevent marine accidents. Furthermore, sensitivity analysis was performed for testing reliability of the experts' evaluation and being clear certainty of the main results/findings in the DEMATEL method. It is found that expert considerations to the casual relationship between human factors are objective and sufficient. The findings of this article provide a critical overview of the research literature on the development of preventive measures for policy makers, shipping companies and port authorities.

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Introduction

Shipping, as a massive international mode of transportation, is becoming increasingly important to global trade and economic development. This increase in marine operations raises the risk of potential ship accident. It is defined by IMO as death, serious injury or loss of a person from ship, loss or abandonment of a ship, material damage to a ship, contact with land, collision of ships and severe damage to the environment directly in connection with the operation of a ship (International Maritime Organization, 2008). Studies on marine accidents indicate that over 75 % of marine accidents occur due to human error (Berg et al., 2013; Wróbel, 2021). Despite building ships equipped with modern technology and setting new rules and regulations to increase the safety, marine accidents still occur (Erol & Başar, 2015). As effects of accidents may be catastrophic to environment, economy and lives, quantification of human error contribution which is accepted as the main cause of marine accidents is of vital importance for maritime domain. As the industry adopts to the autonomous vessels which is expected to reduce human intervention to maritime transportation, the topic is particularly crucial to examine in the maritime domain recently (Hoem et al., 2019). The main goal of the autonomous ships is announced as improving the maritime transportation safety by means of reducing human error, which indicates importance of the subject (Ahvenjärvi, 2016). Although autonomous ships can navigate safely in open seas, it is thought that navigation in confined waters will need human support for safe navigation.

Navigation is more complicated at confined waters such as canals, rivers, straits and port areas due to difficulties like heavy ship traffic, proximity to dangers and many other complications (van Westrenen, 1995). The rapid expansion of marine transportation, particularly in recent decades, has resulted in congested marine traffic in confined waters (Wu et al., 2020). Growing ship size has also been highlighted as a navigation and manoeuvring difficulty in previous research (van Westrenen, 1996). All these complications are pointing out that support of professionals who are aware of the dangers at local waterways is crucial for safe navigation of ships at confined waters. Maritime pilotage is a profession that provides this support to the ships navigating at confined waters in order to ensure safe passage of ships from these dangerous waterways. With the knowledge and experience it provides, pilotage has vital role on risk reducing during passage from dangerous fairways (Uğurlu et al., 2017).

Many researchers studied ship accidents and their reasons in the last decades. Mutual point of these studies is presenting human error as the major reason of maritime accidents. The most popular technique used in maritime domain for human error identification is the Human Factors Analysis and Classification System (HFACS) which is developed from Reason's Swiss Cheese model (B. Wu et al., 2022). Chen et al. (2013) proposed HFACS for maritime accidents (HFACS-MA) in line with HFACS, Hawkins' SHEL and Reasons' Swiss Cheese Models. HFACS has four main causal categories for classifying human errors; unsafe acts, preconditions for unsafe acts, unsafe supervision and organizational influences. In the framework proposed in their study, external factors are integrated in the main causal factors of traditional HFACS. In the study, authors analysed capsizing reasons of a ship in the port and found out that preconditions are the primary factors with 34.8% frequency which are followed by unsafe acts (26.1%), unsafe supervisions (21.7%), organizational influences (13%), and external factors (4.3%), respectively (Chen et al., 2013). Uğurlu et al. (2018) recommended HFACS for passenger vessel accidents (HFACS-PV), and examined 70 ship collision and contact accidents involving passenger vessels by using their proposed framework. In the framework, operational conditions are integrated in the main four causal categories of HFACS structure. Findings of the study reveal that unsafe acts are the primary factors contributing to accident occurrence with 35.01% frequency which are followed by preconditions for unsafe acts (30.37%), operational conditions (19.92%), organizational influences (11.21%), and unsafe supervision (3.48%), respectively (Uğurlu et al., 2018). Yıldırım et al. (2019) studied collision and grounding incidents by using HFACS. This study demonstrated that decision errors, resource management failures, violations, skill-based errors and communication errors are the main human errors leading to accidents (Yıldırım et al., 2019). Erdem & Akyuz (2021) used Success Likelihood Index Method (SLIM) and interval type-2 fuzzy sets (IT2FSs) to evaluate the potential impact of human errors in maritime domain. Due to the significant threats to the marine environment, a loading procedure onboard a containership was evaluated in the study. The results revealed that safety culture, fatigue, time limitation, and experience are deeply influential on crew performance (Erdem & Akyuz, 2021). Uflaz et al. (2022) outlines the principles of fuzzy-based shipboard operation human reliability analysis (SOHRA), which is used to quantitatively assess human error during ship preparation procedures for navigation. The overall human error probability for preparing the ship for sailing is found to be 1.49E-01. It is stated that the study will provide practical contributions to shore-based safety professionals, ship managers and masters of the ship (Uflaz et al., 2022).

Though human factors in maritime accidents are studied frequently, effects of human factors on accidents under pilotage are very limited. For instance, Graziano et al. (2016) classified human errors in collision and grounding accidents by the Technique for the Retrospective and Predictive Analysis of Cognitive Errors (TRACEr) taxonomy. A total of 52 accident reports involving 64 ships were analysed and 289 obtained task errors were classified by TRACEr. The results revealed that 28.7% of the task errors are classified under navigation tasks and pilotage errors has the highest share with 43.6% among navigation subtasks. This result indicates that 12.5% of the task errors are related to pilotage activities among the grand total (Graziano et al., 2016). Park et al. (2019) investigated the relation between pilots' age and accidents under pilotage. The study indicated that after pilot retirement age was increased from 65 to 68, ship accidents under pilotage increased seriously. They also underlined that fatigue is one of the main contributors to human errors thus, resting hours of the pilots should be rescheduled to reduce the fatigue (Park et al., 2019). Ernstsen & Nazir (2020) studied a systematic human error reduction and prediction approach (SHERPA) to investigate the types of errors and error remedies encountered in pilotage operations. Analysis of SHERPA results revealed that action omission is the most frequent human error contributing to accidents where communication error is the second most frequent one (Ernstsen & Nazir, 2020).

International Group of P&I Clubs (IGP&I) published a report about P&I claims involving vessels under pilotage between years 1999-2019 (IGP&I, 2020). As reported, there are 1,046 incidents resulting liabilities in excess of 1.82 billion USD. These numbers indicate that ship accidents which occur under pilotage has an average liability of 1.74 million USD per incident, demonstrating fatality of the results in case of failure at maintaining safety of navigation during pilotage waters. Incident categories and statistics for the years between 1999-2018 are shown in Table 1, however, since the data for 2019 are limited, they are not included in the Table 1. The report also points out that general cause of the incidents is insufficient performance of the bridge team and suggests that effective master-pilot exchange and good bridge resource management are crucial for the safe navigation under pilotage. At the loss prevention poster published by West of England P&I Club for navigation with pilot, it is highlighted that navigation in pilotage waters is mutual task of the bridge team and the pilot (IGP&I, 2020). Potential risks listed in the poster while navigating with pilot are failures at communication, cooperation and situation awareness which are connected to human element. All this information leads us conclude that the majority of accidents under pilotage are caused by human error which is also the major cause for shipping accidents in general (Erol & Başar, 2015; Macrae, 2009; Sánchez-Beaskoetxea et al., 2021).

Table 1. Categorization of the incidents occurred during pilotage (IGP&I, 2020)

Year	No. of Incidents	Total Coast	Average Cost Per Incident	Allision & Contact	Collision	Grounding	Navigation
1999	33	$21,761,748	$659,447	26	6	1	0
2000	47	$35,371,471	$752,584	29	13	5	0
2001	70	$51,090,973	$729,871	45	21	4	0
2002	52	$41,662,008	$801,192	38	9	4	1
2003	56	$106,305,096	$1,898,305	35	16	3	2
2004	59	$76,596,850	$1,298,252	29	20	10	0
2005	46	$39,563,866	$860,084	20	20	5	1
2006	54	$112,306,540	$2,079,751	29	20	5	0
2007	57	$306,538,481	$5,377,868	30	20	6	1
2008	57	$50,811,280	$891,426	31	22	4	0
2009	38	$149,212,660	$3,926,649	26	10	2	0
2010	32	$70,436,063	$2,201,127	23	7	2	0
2011	59	$76,077,997	$1,271,310	32	25	2	0
2012	74	$130,646,688	$1,765,496	49	21	4	0
2013	42	$107,118,832	$2,550,448	25	13	4	0
2014	79	$144,241,993	$1,825,848	39	32	7	1
2015	70	$134,125,800	$1,916,083	40	25	4	1
2016	42	$66,593,613	$1,585,562	27	9	6	0
2017	45	$42,425,808	$942,796	32	10	2	1
2018	34	$58,769,271	$1,728,507	25	8	1	0
Totals	1,046	$1,821,657,039	$1,741,545	630	327	81	8

In all the studies mentioned above, realizing that human factors contributing to maritime accidents during pilotage are not studied comprehensively is our main motivation to conduct this study. Within this scope, critical factors and causal relations among them that cause maritime accidents with presence of pilot are determined by using multi-criteria decision-making methods (MCDM), Decision Making Trial and Evaluation Laboratory (DEMATEL). Moreover, preventive actions are proposed based on determined critical factors.

DEMATEL Method

DEMATEL (The Decision Making Trial and Evaluation Laboratory) is one of the Multi Criteria Decision Making (MCDM) methods, and was developed in 1972 by the Battelle Memorial Institute of Geneva Research Center to visualize the structure of complicated causal relationships through matrixes or digraphs (Gabus & Fontela, 1972). In the terms of the structural modelling approach, DEMATEL method has many advantages and capabilities such as analysing causal relations between criteria of the system, converting the interdependency relations into cause and effect group, detecting most critical criteria, reflecting relative relation of the criteria, and so on (Si et al., 2018). Thanks to its advantages and capabilities, DEMATEL method is used to solve complicated problem in many application areas such as engineering (Zhang vd., 2020), social science (Demirci & Uygur, 2021), and energy (Büyüközkan & Güleryüz, 2016).

The main steps applied of the DEMATEL method are introduced as follows and its flowchart is presented in Figure 1.

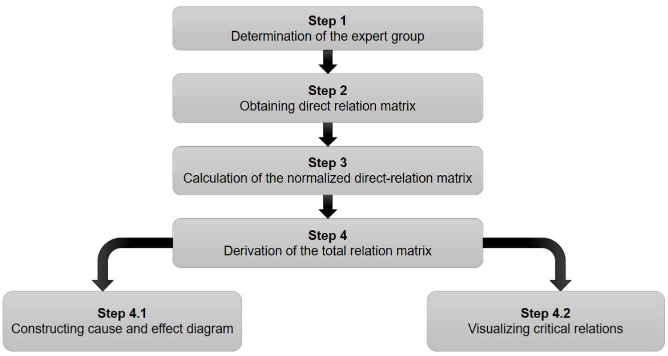

Figure 1. Flowchart of the DEMATEL method

Step 1: Determination of the Expert Group

This step includes the determination of experts who know the problem well and can evaluate it from different perspectives for problem-solving. These experts should be people who have experienced or observed the problem.

Step 2: Obtaining Direct Relation Matrix (A)

Secondly, each experts' evaluation for criteria comparison is obtained by using scale between 0 and 4, presented in Table 2 (Akyuz & Celik, 2015). Arithmetic means of expert evaluations for each comparison which shown in Eq. (1) is used as the direct relation matrix.

Table 2. Corresponding relationship of evaluation criteria

Linguistic terms	Numerical values
No impact (No)	0
Very low impact (VL)	1
Low impact (L)	2
High impact (H)	3
Very high impact (VH)	4

$$A = \begin{bmatrix} 0 & a_{12} & \cdots & a_{1n} \\ a_{21} & 0 & \cdots & a_{2n} \\ \vdots & a_{32} & 0 & a_{3n} \\ a_{n1} & a_{42} & \cdots & 0 \end{bmatrix} \quad (1)$$

Step 3: Calculation of the Normalized Direct-Relation Matrix (S)

The normalization of matrix A can be achieved with following Eq. (2) and Eq. (3), respectively (Si et al., 2018). Thus, each element of matrix S should be in the range of 0 to 1.

$$K = \frac{1}{max_{1 \le i \le n}} \sum_{j=1}^{n} a_{ij} \quad (2)$$

$$S = K \times A \quad (3)$$

Step 4: Derivation of the Total Relation Matrix (M)

Total relation matrix M is derived using Eq. (4) from S. In this Equations, I denotes the identity matrix (Soner, 2021).

$$M = S(I - S)^{-1} \quad (4)$$

Step 4.1: Constructing cause and effect diagram

In this step, the sum of row and columns of total relation matrix are calculated by using Eq. (5) and Eq. (6), respectively (Xia et al., 2015). In these equations, while r_i denotes all direct and indirect influence given by criterion i to all other parameters, c_j denotes the degree of influenced impact.

$$r_i = \left[\sum_{j=1}^{n} m_{ij}\right]_{nx1} \quad (5)$$

$$c_j = \left[\sum_{i=1}^{n} m_{ij}\right]_{1xn} \quad (6)$$

$$M = m_{ij}, \quad i,j = 1,2, \cdots n.$$

where r_i denotes sum of rows in total relation matrix and refers all direct and indirect influence given by criterion *i* to all other criteria. Similarly, c_j denotes sum of columns in total relation matrix and refers all direct and indirect influence received by criterion *j* from others.

To construct cause – effect diagram, $r_i + c_j$ and $r_i - c_j$ values are calculated. In the diagram, these values define coordinates of criteria and refer horizontal and vertical axis values, respectively. $r_i - c_j$ value is named as relation and if relation value is positive, criterion j can be grouped into cause group, otherwise it can be grouped into effect group. When $i=j$, $r_i + c_j$ value shows importance of criterion and is called as prominence. While the visualization of relation among criteria with cause-effect diagram, all relation which have indirect and direct effect between criteria are taken into account.

Step 4.2: Visualizing critical relations

The total relation matrix contains all direct and indirect relations between criteria. For this reason, with the aim of filtering some minor effects between criteria and exploring critical relations between criteria, the threshold value *(θ)* can be defined with summation of standard deviation and mean of m_{ij} figures from total relation matrix (Xia et al., 2015). The values over threshold value shows critical relations. These values present effects of criteria on each other.

Application and Results

Over one-third of the total human population lives within 60 miles of coastal areas. For this reason, maritime accidents happened in near coastal areas excessively affect people, environment and property. To prevent accidents in coastal areas, many preventive measures has been taken by government and private sector organisations. Some of the major and well-known measures are pilotage service, navigation aids, vessel traffic service (VTS), traffic separation scheme. Among all these measures, the pilotage service stands out as the first and most effective measure in terms of directly intervening in the ship's manoeuvre to prevent the accident. Therefore, when examining the human factor in accidents that occurred under pilotage, the relationship between pilots and ship crew should be considered. In this study, to begin the analysis, critical human-related criteria that have vital role in maritime accident under pilotage area were determined with the help of accident investigation reports published by GISIS and expert opinions. Criteria determined are presented in Table 3.

In the first step, initially, professionals who are considered to be directly influential in ship control and have at least 15 years of experience were selected as experts. Information about the experts consulted in this study is given Table 4.

Table 3. Human criteria

Codes	Criteria
C_1	Master Experience
C_2	Pilot Experience
C_3	Bridge Team Management
C_4	Maneuvering Team
C_5	Communication
C_6	Fatigue
C_7	Understanding & Application of Instructions
C_8	Crew Training

Table 4. Information about the experts

Experts	Competency	Current Position	Experience in Year
Expert 1	Oceangoing Master	Master	15 Years
Expert 2	Oceangoing Master	Pilot	17 Years
Expert 3	Oceangoing Master	Harbour Master	21 Years
Expert 4	Oceangoing Chief Officer	Port Captain	18 Years
Expert 5	Oceangoing Chief Officer	VTS Operator	15 Years

Table 5. Direct-relation matrix

Criteria	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
C_1	0.000	3.000	4.000	3.800	4.000	4.000	4.000	4.000
C_2	3.200	0.000	3.800	3.200	3.600	4.000	3.600	2.200
C_3	3.800	3.400	0.000	3.400	4.000	3.800	4.000	3.400
C_4	4.000	3.400	2.800	0.000	4.000	3.800	4.000	3.800
C_5	3.600	3.400	4.000	4.000	0.000	4.000	4.000	3.000
C_6	3.600	3.400	4.000	4.000	4.000	0.000	4.000	3.400
C_7	3.600	2.800	4.000	4.000	3.000	3.000	0.000	3.400
C_8	4.000	3.400	4.000	4.000	3.600	4.000	4.000	0.000

Table 6. Normalized direct relation matrix

Criteria	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
C_1	0.000	0.111	0.148	0.141	0.148	0.148	0.148	0.148
C_2	0.119	0.000	0.141	0.119	0.133	0.148	0.133	0.081
C_3	0.141	0.126	0.000	0.126	0.148	0.141	0.148	0.126
C_4	0.148	0.126	0.104	0.000	0.148	0.141	0.148	0.141
C_5	0.133	0.126	0.148	0.148	0.000	0.148	0.148	0.111
C_6	0.133	0.126	0.148	0.148	0.148	0.000	0.148	0.126
C_7	0.133	0.104	0.148	0.148	0.111	0.111	0.000	0.126
C_8	0.148	0.126	0.148	0.148	0.133	0.148	0.148	0.000

Table 7. Total direct-relation matrix

Criteria	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
C_1	2.382	2.231	**2.572**	**2.557**	2.541	**2.567**	**2.657**	2.307
C_2	2.226	1.897	2.298	2.271	2.265	2.299	2.368	2.014
C_3	2.422	2.168	2.358	2.460	2.457	2.476	**2.569**	2.213
C_4	2.430	2.170	2.455	2.350	2.459	2.478	**2.570**	2.226
C_5	2.431	2.181	2.501	2.491	2.343	2.496	**2.584**	2.214
C_6	2.463	2.210	2.535	2.525	2.505	2.400	**2.618**	2.256
C_7	2.262	2.012	2.328	2.319	2.272	2.295	2.275	2.072
C_8	2.524	2.254	**2.586**	**2.575**	2.543	**2.580**	**2.671**	2.190

To solve complex relations among criteria presented in Table 3, experts were asked to compare these criteria by using the linguistic terms in Table 2. After the comparison, direct relation matrix in Table 5 is achieved by calculating the average of each experts' opinion (Step 2).

Normalization of the direct-relation matrix, Step 3, Eq. (2) and Eq. (3) have been used respectively and normalized direct-relation matrix is presented in Table 6.

To obtain total relation matrix, as the Step 4, Eq. (4) have been utilized and the matrix presented in Table 7. Threshold value is calculated from this matrix (Step 4.1). In this matrix, mean of the all elements and standard deviation are calculated as 2.379 and 0.169, respectively. Threshold value (2.548) has been determined by adding one standard deviation to mean of elements. In Table 7, bold numbers indicate elements over threshold value and refer critical relations. These relations are visualized with the help of chord diagram and presented in Figure 2. The chord diagram is built by using "*circlize*" package in R programming language.

In the application of the Step 4.2, with the help of Eq. (5) and Eq. (6), relation (r_i - c_j) and prominence (r_i + c_j) values has been derived in Table 8 and thus, DEMATEL diagram has been constructed as shown in Figure 3. In this figure, red and green points denote cause and effect criteria, respectively.

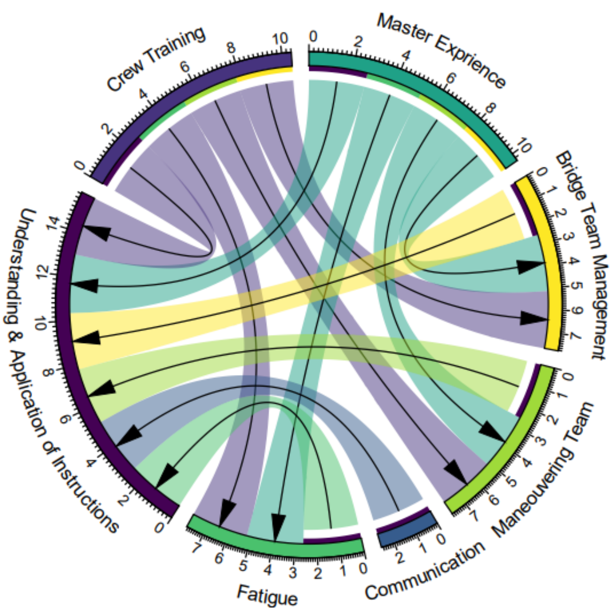

Figure 2. Critical relation between criteria

Table 8. Values of r_i, c_j, $r_i + c_j$ and $r_i - c_j$.

Criteria	$\tilde{r}_i$	$\tilde{c}_j$	$\tilde{r}_i + \tilde{c}_j$	$\tilde{r}_i - \tilde{c}_j$
C_1	19.814	19.141	38.955	0.673
C_2	17.640	17.123	34.762	0.517
C_3	19.123	19.633	38.756	-0.510
C_4	19.138	19.548	38.687	-0.410
C_5	19.241	19.385	38.626	-0.144
C_6	19.511	19.592	39.103	-0.081
C_7	17.836	20.311	38.147	-2.476
C_8	19.923	17.492	37.416	2.431

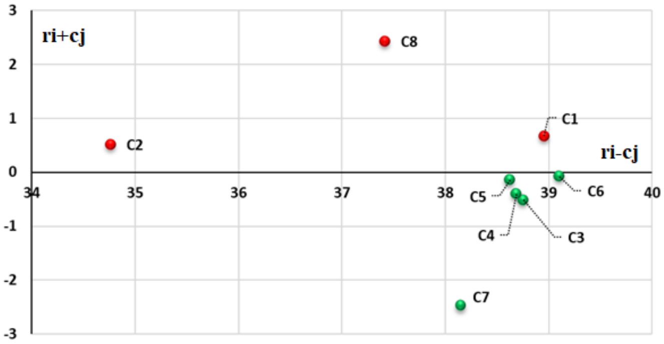

Figure 3. The cause-effect relation diagram

Sensitivity Analysis

Testing reliability of the experts' evaluation and being clear certainty of the main results/findings in the DEMATEL method, sensitivity analysis is conducted. Sensitivity analysis makes it possible to verify whether the results of the DEMATEL are varied by experts' evaluation (Seker & Zavadskas, 2017). In the analysis, experts' evaluations are weighted within the various scenarios in order to detect the effect of criteria compared by experts on the results. According to six scenarios given in Table 9, firstly, equal weights (Scenario 1) are given for each experts' evaluation as applied in Step 1. Then, different weights are given for each expert in the five scenarios to analyse the evaluations how much effect to causal relation between criteria. With the given weights in each scenario, direct relation matrix is calculated by using weighted arithmetic mean.

Afterwards, other steps explained in the section 2 are applied in MS Excel to obtain final DEMATEL results, ($r_i + c_j$) and ($r_i - c_j$) values. Obtained results are given in Table 10 and presented in Figure 4.

The results obtained from the sensitivity analysis for each scenario show that the overall effect on the cause-and-effect criteria remained the same, even if the experts' evaluations were weighted differently. The fact that the lines connecting the criteria are parallel or overlapped for each scenario in Figure 3 shows that the experts are of the similar or same opinion in pair-wise comparison of the criteria. As a result, the approaches of experts to the causal relationship between human factors are unbiased and adequate for this study.

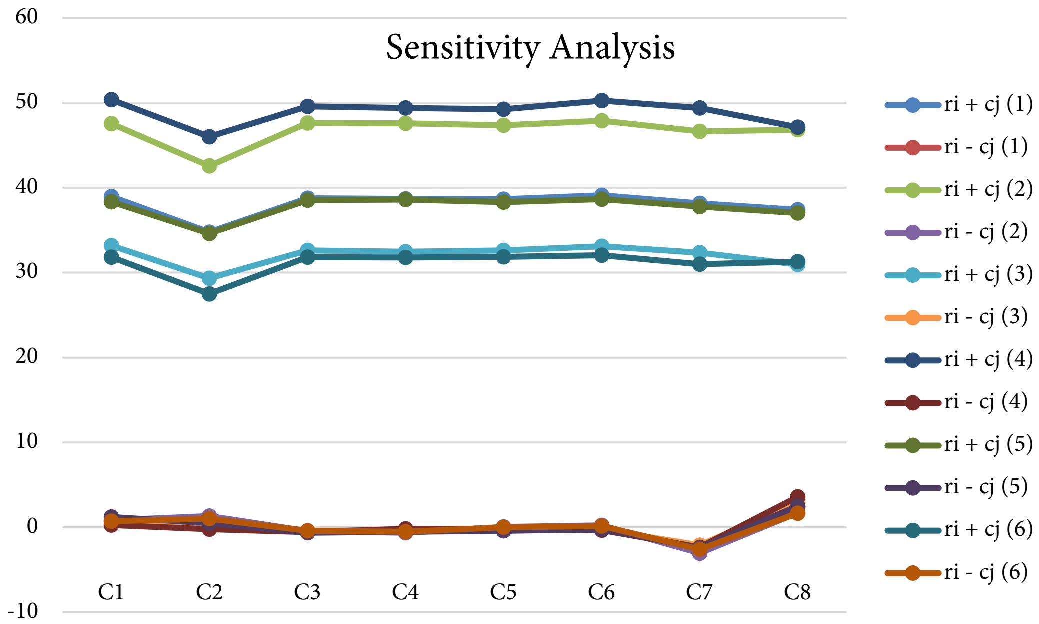

Figure 4. Cause and Effect diagram of sensitivity analysis for each scenario

Table 9. Given weights in each scenario

Experts	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
Expert 1	0.20	0.10	0.30	0.25	0.20	0.15
Expert 2	0.20	0.15	0.10	0.30	0.25	0.20
Expert 3	0.20	0.20	0.15	0.10	0.30	0.25
Expert 4	0.20	0.25	0.20	0.15	0.10	0.30
Expert 5	0.20	0.30	0.25	0.20	0.15	0.10

Table 10. ($r_i + c_j$) and ($r_i - c_j$) values obtained in each scenario

Scenario 1		Scenario 2		Scenario 3		Scenario 4		Scenario 5		Scenario 6	
$r_i + c_j$	$r_i - c_j$	$r_i + c_j$	$r_i - c_j$	$r_i + c_j$	$r_i - c_j$	$r_i + c_j$	$r_i - c_j$	$r_i + c_j$	$r_i - c_j$	$r_i + c_j$	$r_i - c_j$
38.955	0.673	47.527	0.834	33.178	0.333	50.362	0.273	38.355	1.199	31.803	0.710
34.762	0.517	42.560	1.330	29.337	-0.042	46.008	-0.211	34.611	0.504	27.498	1.012
38.756	-0.510	47.632	-0.458	32.600	-0.497	49.579	-0.576	38.546	-0.633	31.813	-0.414
38.687	-0.410	47.573	-0.625	32.463	-0.207	49.376	-0.173	38.611	-0.529	31.774	-0.517
38.626	-0.144	47.360	0.023	32.607	-0.144	49.235	-0.182	38.313	-0.411	31.860	0.000
39.103	-0.081	47.887	0.232	33.111	-0.246	50.244	-0.345	38.653	-0.213	32.045	0.155
38.147	-2.476	46.625	-3.042	32.337	-2.106	49.370	-2.363	37.779	-2.414	31.003	-2.593
37.416	2.431	46.837	1.706	30.948	2.908	47.109	3.577	37.039	2.498	31.292	1.646

Findings

The significant relationships of each factor are explained using a cause-effect diagram which created according to Table 8. The findings obtained from the model created by using expert opinions are divided into two different groups as follows; cause and effect factors.

Cause Factors

In order to clearly assess the most common and critical human factors on accidents during navigation under pilotage, it is essential to focus on the cause factors that have positive value of $\tilde{r}_i - \tilde{c}_j$. As shown in cause-effect diagram (Fig. 1), C_1, C_2 and C_8 are in cause group. In cause group, C_8 (Crew Training) has the highest $\tilde{r}_i - \tilde{c}_j$ value (2.43) among the all factors. This means that C_8 has a more significant impact on the whole of the process. Furthermore, C_8 has the highest $\tilde{r}_i$ value (19.92) among the causal factors from the point of influential impact degree. Following that, C_1 (Master Experience) has the second highest $\tilde{r}_i - \tilde{c}_j$ value (0.67) and is the second most important causal factor among all factors. C_1 has the second highest degree of influential impact ($\tilde{r}_i$) value which is 19.81. Therefore, it has a great influence on the entire process. Also, C_8 and C_1 have critical effect on C_3, C_4, C_6, and C_7. Moreover, C_2 is found as the third most critical factor which means that it had a considerable influence on the entire process ($\tilde{r}_i - \tilde{c}_j$ = 0.52).

Effect Factors

In Figure 1, the factors below the horizontal x-axis are considered as the effect group. As can be clearly seen from Figure 1, it shows that influential factors can be easily affected by other factors. According to the cause-effect diagram, C_6 (Fatigue) has the highest $\tilde{r}_i + \tilde{c}_j$ value (39.10) among the effect factors. In addition, $\tilde{r}_i - \tilde{c}_j$ value of C_6 is very high (-0.08). This means that C_6 is the least affected factor among the whole process and has significant effect on the other factors. Moreover, it has the highest degree of influential impact index ($\tilde{r}_i = 19.51$). Considering $\tilde{r}_i + \tilde{c}_j$ value, the other parameters are sorted by C_3, C_4, C_5 and C_7, respectively and they had also great impacts on the entire process as effect factors. Furthermore, C_7 (understanding & application of instructions) has the lowest $\tilde{r}_i - \tilde{c}_j$ value (-2.48) and this means that C_7 is easily affected from other factors. Specifically, C_7 is critically affected by C_1, C_3, C_4, C_5, C_6 and C_8. It is also observed that Bridge Team Management (C_3) has the second highest $\tilde{r}_i + \tilde{c}_j$ value (38.76) among the other effect factors.

Discussion

The human factor is accepted the primary cause of the majority of marine accidents. The human factor, which accounts for 75-96% of maritime accidents involving modern ships, is a major issue that must be recognized and addressed. One of the most difficult tasks for investigators is determining the reasons of human error because the role of human influence in these errors is revealed by the combination of many parameters that are ignored after an accident. However, it is possible to determine the source of human errors that occur in many accidents by analysing them with the right people who are experts in their fields. That is why this study is of essential importance. In this study, the human factor in accidents that may occur with pilot onboard was investigated by using the DEMATEL method by taking expert opinions. In the report published by the Canadian Transportation and Safety Board (CTSB), it is seen that 273 incidents that occurred in the Canadian pilotage waters from 1987 to 1992. CTSB also reported that 42% of incidents involving misunderstandings or lack of communication between the pilot and the captain or watch officer. Hetherington et al. (2006) stated that the term "misunderstanding" represents a lack of situation awareness and poor team working as well as inadequate communication (Hetherington et al., 2006). In this context, many companies have implemented Bridge Team Management (BTM) courses because they are recommended by the IMO. Although BTM courses are compulsory for deck officers, there is still lack of skills, which increases the possible accident risks. According to MSC-MEPC.2/Circ.13, the rules and regulations that implemented to seafarers should be simple, clear and comprehensive.

The results also emphasize the significance of collaboration once again. Crew training, master and pilot experiences were found to be associated with the occurrence of marine accidents with presence of pilot. In order to minimize the effect of such human factors on the occurrence of accidents, it is necessary to continually improve the quality of maritime training and standards to meet the constantly changing needs, and to provide regular knowledge by updated trainings to all crew. Akyuz & Celik (2014) emphasized the need for routine and special training to lead personnel and all crew members in order to improve ship safety standards. Additionally, it is recommended that the safety training officer on board implement an effective training program coordinated with the Master (Akyuz & Celik, 2014). The significance of pilot experience is found to be low in maritime accidents with presence of pilot, however errors originated by pilots are considered to be caused by fatigue and communication failures instead of pilot experience. Fatigue is not only a problem to be prevented in order to improve the individual well-being of seafarers but it is also one of the most important human factors that can increase the risk of accidents and fatal disasters. Crew member performances are affected by fatigue due to its effects of reducing situation awareness, causing planning deficits/errors, leading to inability to adapt to new knowledge, frequent forgetfulness and concentration difficulties. Moreover, crew performance is directly related to management policies, cultural factors, experience, training and so on (MSC-MEPC.2-Circ.13, 2013). Rothblum et al. (2002) stated that human factors such as fatigue, stress, health, situation awareness, and cultural diversity might cause to unsafe situations (Rothblum et al., 2002). Moreover, extended hours on duty are contributed to marine accidents that could be attributable to fatigue (Raby & McCallum, 1997). The authors also examined 98 ship casualty reports and found that fatigue was effective in 23% of cases. One of the most common human factors is seafarer fatigue. Several factors such as working hours, extended work, the short period of rest between working hours and excessive workload can contribute to fatigue (Smith et al., 2007). Therefore, work rest hours of both pilot and ship crew should be reconsidered and revised in order to provide effective work/rest periods and minimize fatigue. In order to reduce the possibility of human error as much as possible, preventive actions should be proposed and discussed with all stakeholders in light of the findings. Our observations are in agreement with previous studies regarding the human factor is an important factor in marine accidents (Trucco et al., 2008; Tzannatos, 2010; Fan et al., 2018).

Conclusion

Ship accidents have caused global ecological, environmental, and economic problems, posing a hazard to human life. Especially, accidents in confined waters in recent years (Ever Given) have seriously damaged global trade and powerful economies around the world. Therefore, to prevent accidents in these areas, many preventive measures has been taken by government and private sector organisations. Although pilotage is taken as one of the major preventive measures in coastal areas, accidents still occur in coastal areas such as canal, strait, and port area. In addition, preventive measures are taken with national and international rules and regulations in order to minimize the effect of other human-induced factors that have a significant impact on accidents.

In present study, the DEMATEL method was used to solve the complex relationships among human factors considered as affecting accidents with presence of pilot at confined waters. As a result of the study, it is found that master experience (C_1) and crew training (C_8) have influential role on the other factors. Furthermore, understanding & application of instructions (C_7) is most affected by other factors. As an interesting result of the study is that pilot experience (C_2) is among the influential factors and is less impressive than the others. This imply that the main cause of maritime accidents is caused by human factors originating from the ship and points out the importance of seafarer's competences and skills in safe ship management. To ensure the reliability of the obtained results, sensitivity analysis was performed and it is revealed that the experts' evaluations on the human factors are consistent in case of different weights too.

With the help of DEMATEL results, we believe that the maritime stakeholders can determine their policies for the prevention of accidents and regulate relation between critical human factors. In future studies, the scope of the study can be expanded by considering the environmental and ship-related factors that are thought to have an effect on the maritime accident under pilotage.

Compliance With Ethical Standards

Authors' Contributions

SMED: Conceptualization, Methodology, Software, Original draft preparation.

RC: Conceptualization, Methodology, Original draft preparation.

HE: Supervision, Writing- Reviewing and Editing.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Ahvenjärvi, S. (2016). The human element and autonomous ships. *International Journal on Marine Navigation and Safety of Sea Transportation*, *10*(3), 517-521. https://doi.org/10.12716/1001.10.03.18

Akyuz, E., & Celik, E. (2015). A fuzzy DEMATEL method to evaluate critical operational hazards during gas freeing process in crude oil tankers. *Journal of Loss Prevention in the Process Industries*, *38*, 243-253. https://doi.org/10.1016/j.jlp.2015.10.006

Akyuz, E., & Celik, M. (2014). Utilisation of cognitive map in modelling human error in marine accident analysis and prevention. *Safety Science*, *70*, 19-28. https://doi.org/10.1016/j.ssci.2014.05.004

Berg, N., Storgård, J., & Lappalainen, J. (2013). *The impact of ship crews on maritime safety*. University of Turku, Centre for Maritime Studies.

Büyüközkan, G., & Güleryüz, S. (2016). An integrated DEMATEL-ANP approach for renewable energy resources selection in Turkey. *International Journal of Production Economics*, *182*, 435-448.

Chen, S. -T., Wall, A., Davies, P., Yang, Z., Wang, J., & Chou, Y. -H. (2013). A human and organisational factors (HOFs) analysis method for marine casualties using HFACS-Maritime Accidents (HFACS-MA). *Safety Science*, *60*, 105-114. https://doi.org/10.1016/j.ssci.2013.06.009

Demirci, S. M. E., & Uygur, S. (2021). *DEMATEL yöntemi ile denizcilik mesleği seçimini etkileyen kriterlerinin incelenmesi* [Investigation of the criteria affecting the selection of maritime profession with the DEMATEL method]. *Journal of Marine and Engineering Technology*, *1*(2), 68-76.

Erdem, P., & Akyuz, E. (2021). An interval type-2 fuzzy SLIM approach to predict human error in maritime transportation. *Ocean Engineering*, *232*, 109161. https://doi.org/10.1016/j.oceaneng.2021.109161

Ernstsen, J., & Nazir, S. (2020). Performance assessment in full-scale simulators – A case of maritime pilotage operations. *Safety Science*, *129*, 104775. https://doi.org/10.1016/j.ssci.2020.104775

Erol, S., & Başar, E. (2015). The analysis of ship accident occurred in Turkish search and rescue area by using decision tree. *Maritime Policy & Management*, *42*(4), 377-388. https://doi.org/10.1080/03088839.2013.870357

Fan, S., Zhang, J., Blanco-Davis, E., Yang, Z., Wang, J., & Yan, X. (2018). Effects of seafarers' emotion on human performance using bridge simulation. *Ocean Engineering*, *170*, 111-119. https://doi.org/10.1016/j.oceaneng.2018.10.021

Gabus, A., & Fontela, E. (1972). World problems, an invitation to further thought within the framework of DEMATEL. *Battelle Geneva Research Center, Geneva, Switzerland*, 1-8.

Graziano, A., Teixeira, A. P., & Guedes Soares, C. (2016). Classification of human errors in grounding and collision accidents using the TRACEr taxonomy. *Safety Science*, *86*, 245-257. https://doi.org/10.1016/j.ssci.2016.02.026

Hetherington, C., Flin, R., & Mearns, K. (2006). Safety in shipping: The human element. *Journal of safety research*, *37*(4), 401-411.

Hoem, Å. S, Fjortoft, K. E., & Rødseth, Ø. J. (2019). Addressing the accidental risks of maritime transportation: Could Autonomous shipping technology improve the statistics? *International Journal on Marine Navigation and Safety of Sea Transportation*, *13*(3), 487-494. https://doi.org/10.12716/1001.13.03.01

IGP&I. (2020). *Report on P&I claims involving vessels under pilotage 1999-2019*. International Group of P&I Clubs.

International Maritime Organization (IMO). (2008). *Casualty Investigation Code* (s. 3).

Macrae, C. (2009). Human factors at sea: Common patterns of error in groundings and collisions. *Maritime Policy & Management*, *36*(1), 21-38. https://doi.org/10.1080/03088830802652262

MSC-MEPC.2-Circ.13, I. (2013). *Guidelines for the application of the human element analysing process (heap) to the IMO rule-making process.*

Park, Y. A., Yip, T. L., & Park, H. G. (2019). An analysis of pilotage marine accidents in Korea. *The Asian Journal of Shipping and Logistics*, *35*(1), 49-54. https://doi.org/10.1016/j.ajsl.2019.03.007

Raby, M., & McCallum, M. C. (1997). Procedures for investigating and reporting fatigue contributions to marine casualties. *Proceedings of the Human Factors and Ergonomics Society Annual Meeting*, *41*(2), 988-992.

Rothblum, A. M., Wheal, D., Withington, S., Shappell, S. A., Wiegmann, D. A., Boehm, W., & Chaderjian, M. (2002). Human factors in incident investigation and analysis. *Report of Working Group, 2nd International Workshop on Human Factors in Offshore Operations*, Texas, 142p.

Sánchez-Beaskoetxea, J., Basterretxea-Iribar, I., Sotés, I., & Mauri Machado, M. M. (2021). Human error in marine accidents: Is the crew normally to blame? *Maritime Transport Research*, *2*, 100016. https://doi.org/10.1016/j.martra.2021.100016

Seker, S., & Zavadskas, E. K. (2017). Application of fuzzy DEMATEL method for analyzing occupational risks on construction sites. *Sustainability*, *9*(11), 2083. https://doi.org/10.3390/su9112083

Si, S.-L., You, X.-Y., Liu, H.-C., & Zhang, P. (2018). DEMATEL technique: A systematic review of the state-of-the-art literature on methodologies and applications. *Mathematical Problems in Engineering*, *2018*, 3696457. https://doi.org/10.1155/2018/3696457

Smith, A. P., Allen, P. H., & Wadsworth, E. M. (2007). A comparative approach to seafarers' fatigue. *Proceedings of the International Symposium on Maritime Safety, Science and Environmental Protection, Greece*, pp. 1-15.

Soner, O. (2021). Application of fuzzy DEMATEL method for analysing of accidents in enclosed spaces onboard ships. *Ocean Engineering*, *220*, 108507. https://doi.org/10.1016/j.oceaneng.2020.108507

Trucco, P., Cagno, E., Ruggeri, F., & Grande, O. (2008). A Bayesian Belief Network modelling of organisational factors in risk analysis: A case study in maritime transportation. *Reliability Engineering & System Safety*, *93*(6), 845-856. https://doi.org/10.1016/j.ress.2007.03.035

Tzannatos, E. (2010). Human element and accidents in Greek shipping. *The Journal of Navigation*, *63*(1), 119-127. https://doi.org/10.1017/S0373463309990312

Uflaz, E., Celik, E., Aydin, M., Erdem, P., Akyuz, E., Arslan, O., Kurt, R. E., & Turan, O. (2022). An extended human reliability analysing under fuzzy logic environment for ship navigation. *Australian Journal of Maritime & Ocean Affairs*, *In press*. https://doi.org/10.1080/18366503.2022.2025687

Uğurlu, Ö., Kaptan, M., Kum, S., & Yildiz, S. (2017). Pilotage services in Turkey; key issues and ideal pilotage. *Journal of Marine Engineering & Technology*, *16*(2), 51-60. https://doi.org/10.1080/20464177.2016.1262596

Uğurlu, Ö., Yıldız, S., Loughney, S., & Wang, J. (2018). Modified human factor analysis and classification system for passenger vessel accidents (HFACS-PV). *Ocean Engineering*, *161*, 47-61. https://doi.org/10.1016/j.oceaneng.2018.04.086

van Westrenen, F. (1995). Towards a Decision Making Model of River Pilots. *IFAC Proceedings Volumes*, *28*(21), 217-222. https://doi.org/10.1016/S1474-6670(17)46728-0

van Westrenen, F. (1996). A framework for a decision model of river-pilots based on their workload. *International Journal of Industrial Ergonomics*, *17*(5), 411-418. https://doi.org/10.1016/0169-8141(94)00118-9

Wróbel, K. (2021). Searching for the origins of the myth: 80% human error impact on maritime safety. *Reliability Engineering & System Safety*, *216*, 107942. https://doi.org/10.1016/j.ress.2021.107942

Wu, B., Yip, T. L., Yan, X., & Guedes Soares, C. (2022). Review of techniques and challenges of human and organizational factors analysis in maritime transportation. *Reliability Engineering & System Safety*, *219*, 108249. https://doi.org/10.1016/j.ress.2021.108249

Wu, L., Jia, S., & Wang, S. (2020). Pilotage planning in seaports. *European Journal of Operational Research*, *287*(1), 90-105. https://doi.org/10.1016/j.ejor.2020.05.009

Xia, X., Govindan, K., & Zhu, Q. (2015). Analyzing internal barriers for automotive parts remanufacturers in China using grey-DEMATEL approach. *Journal of Cleaner Production*, *87*, 811-825. https://doi.org/10.1016/j.jclepro.2014.09.044

Yıldırım, U., Başar, E., & Uğurlu, Ö. (2019). Assessment of collisions and grounding accidents with human factors analysis and classification system (HFACS) and statistical methods. *Safety Science*, *119*, 412-425. https://doi.org/10.1016/j.ssci.2017.09.022

Zhang, X., Ming, X., & Yin, D. (2020). Application of industrial big data for smart manufacturing in product service system based on system engineering using fuzzy DEMATEL. *Journal of Cleaner Production*, *265*, 121863. https://doi.org/10.1016/j.jclepro.2020.121863

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RESEARCH ARTICLE

Blue swimming crab (*Portunus pelagicus*, Linnaeus 1758) capture fishery practices in Tigbauan, Iloilo, central Philippines

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ABSTRACT

Blue swimming crab (BSC) (*Portunus pelagicus*) is an economically significant aquatic species contributing to the Philippine economy. Tigbauan, a coastal municipality in Iloilo, central Philippines, has been practicing BSC capture fishery for decades. However, there is a limited existing study on BSC fishing practices in this particular area. Thus, this study aimed to assess the status of the BSC capture fishery in Tigbauan, Iloilo. A household survey was done in October 2019 for the five crabbing villages (barangays) in the area using a semi-structured questionnaire. Results showed that most crabbers (83.4%) were still living below the country poverty threshold. Although some crabbers were engaged in other livelihood opportunities, the marginal contribution from these earnings to the household income was still inadequate to supplement the fundamental and other family needs. Limiting their chances to other sources of income may be due to their lack of formal education. Fishing gears used by locals were crab pots locally known as *panggal* and crab entangling net or bottom-set gillnet locally known as *pukot*. Crabbing operation varied in each village and was done regularly and even twice a day in peak season time. The instances of catching gravid females were high. Hence, threatening the status of wild stocks. Rampant trawling and dragging activities in the municipality also became a big problem for the crabbers and contributed to the catch decline. Strict implementation of the existing ordinances must be carried out and focused on the biology, seasonality, and stock enhancement to formulate an effective management framework plan.

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Introduction

Crab fishing is one of the primary livelihoods in a coastal community. In the Philippines, as an archipelagic country, crabbers are common to find. Since crabs are among the most vital aquatic resources of the Philippine waters (Camacho & Aypa, 2001), they also provide a family living. Among the commercially significant crab species are the BSCs, scientifically known as *Portunus pelagicus*, which are either sold in the live state or as processed products locally or exported mainly to Taiwan, Hongkong, Japan, and the USA (Camacho & Aypa, 2001). In the Philippines, BSCs constitute a crucial part of the local fisheries production. *P. pelagicus*, over 90% of the crabs landed, was the main species in the country's crab fishery since it started in the 1950s (Ingles, 2004). In the first quarter of 2018, the BSC fisheries production amounted to 5,923.67 metric tons, contributing 0.01% to the total Philippine fisheries production (PSA, 2018a).

BSC fisheries occur throughout the Philippines (BFAR, 2013). The fishing activity of *P. pelagicus* started in the Philippines as early as the 1950s (Mesa et al., 2018). The BSC fishery is a multi-gear activity where crabbers are usually artisanal and capture crabs by gleaning, bamboo trap use, crab lift nets (Romero, 2009; Gadhavi et al., 2013), crab pots, and gill nets (Del Norte-Campo et al., 2004; Ingles, 2004; Germano & Melgo, 2003). The most common artisanal gear used in shallow water is crab pots or *panggal*, but crabbers also use the bottom set gillnets, otter trawl, crab lift net or *bintol*, and push net (Mesa et al., 2018). In the study of Ingles (2004) and Mesa et al. (2018), they have identified eleven different fishing gear types used in catching BSCs in the Western Visayan Sea. Five out of these eleven crab gears are considered as one of the major fishing gears used by crabbers. Gadhavi et al. (2013) also mentioned that a large number of fishers around Sikka, Gulf of Kutch, Gujarat, India is engaged in artisanal crab fishery using spears, traps, and other traditional gears like umbrella net and fence net. Different specifications of crabbing gears and crabbing operations were also discussed by Romero (2009), particularly used as well in the Visayan Sea. Hence, few publications show several fishing gears used in this type of species with its unique specifications and operations depending on the location it is being utilized.

In Tigbauan, a coastal municipality in Iloilo, central Philippines, BSC fishing activities have been practiced for years. This practice provides a livelihood for the crabbers. Accordingly, different fishing gears are used to catch BSC. The fishing gears, gear operations, and fishing practices are not well documented (Pers. Comm. Municipal Aquaculturist, Tigbauan, Iloilo). Thus, this study aimed to assess the BSC capture fishery practices in Tigbauan, Iloilo, central Philippines.

Specifically, this study aimed to;

(1) survey the crabbers' socio-demographic profile, crabbing gears and gears modifications (if there is any), and crabbing practices which emphasized the operation of crabbers with specified gear used;

(2) assess whether the municipal ordinance has regulatory measures regarding BSC fishery management; and

(3) measure the level of crabbers' awareness about the ordinance (if there is any).

Materials and Methods

Study Site and Duration

A household survey (HS) of the various crabbing villages in Tigbauan, Iloilo, central Philippines, was conducted for a month in October 2019. The study covered the five Tigbauan coastal villages engaged in crab fishing: Barroc, Atabayan, Baguingin, Namocon, and Parara Norte (Figure 1).

Data Gathering

Preliminary survey and key informant interview (KII) were carried out with the Municipal Fisheries Officer and the president of the Barangay Fisheries and Aquatic Resources Management Council (BFARMC) in each sampling station to have an overview of the crab fishery in the municipality. After which, a one-on-one HS was done with the crabbers. A semi-structured questionnaire was developed and used to address the objectives of this study. The survey had included individual fishers who engaged in crabbing activities either part-time or full-time, as adopted in the study of de la Cruz et al. (2018), who were available and willing to participate in the study. The survey questions included information on the crabber's socio-economic profile, namely age, sex, civil status, educational attainment, number of dependents, and average monthly income from crabbing. For the gear preference and crabbing practices, each crabber was asked the following: type of gear used or preferred; dimension or number of gear units; soaking time of gears; the frequency of crabbing; bait used, if any; and companion in crabbing. Regarding BSC fishery management awareness, the respondents were asked about what type of problems and concerns related to crabbing experiences, knowledge on any government or non-government regulatory measures to manage the BSC resource, and suggestions to address their issues and concerns and to manage the resources effectively.

Fishing gears used in crabbing and its specification were described and documented.

Data Analysis

A database was made on the socio-demographic profile of the crabbers using IBM SPSS Statistics version 20. The data were tabulated and thoroughly analyzed. Qualitative data were analyzed using descriptive statistics, and mean scores were derived for all quantitative results.

Results and Discussion

Crabber's Socio-Demographic Profile

A total of 24 crabbers were interviewed from five villages of Tigbauan: Parara Norte, Namocon, Baguingin, Atabayan, and Barroc. Only two out of the total interviewed crabbers were not members of the BFARMC. Atabayan village has the highest percentage of crabbers (45.8%), followed by Baguingin (25%), Namocon (16.7%), Parara Norte (8.3%), and Barroc (1%) (Table 1). All crabbers were male, aged from 27 – 72 years old, married with 6-10 household members, and practiced Roman Catholicism. About 79.2% of them resided in the area for more than 30 years. The majority (33.3%) graduated high school, 20.8% attained vocational courses, 4.2% had gone into college, and only 4.2% successfully gained a degree.

Each crabber varied in their years of crab fishing from more than 30 years to just 1 to 5 years of engagement. Half of the total count of crabbers already owned a lot of permanent residents, but half of them were informal settlers (Table 1). Crabbers' houses, made of semi-concrete materials, were provided by electricity from Iloilo Electric Company (ILECO) and available water supply from a deep well or water pump.

Table 1. Crabbers count per village in Tigbauan, Iloilo, Philippines

Village	Crabbers' Count	Percentage Composition (%)
Barroc	1	4.2
Namocon	4	16.7
Atabayan	11	45.8
Parara Norte	2	8.3
Baguingin	6	25
Total	24	100

Most crabbers (54.2%) had an average monthly income of PHP 1,000.00 (US$ 19.46) – PHP 5,000.00 (US$ 97.28). About 29.2% of the respondents had an income of PHP 6,000.00 (US$ 116.74) – PHP 10,000.00 (US$ 194.57) and 8.3% earned PHP 11,000.00 (US$ 214.03) – PHP 15,000.00 (US$ 219.85) monthly. Only 8.4% of the crabbers earned an average monthly income of more than PHP 26,000.00 (US$ 505.88) (Table 2). During the 1st semester of 2018, a family of five needed around PHP 10,481 (US$ 03.93) monthly to meet their minimum basic food and non-food needs. This represents an increase of about 11% from the 1st Semester of 2015 to the 1st half of 2018. On the other hand, a family of five needed around PHP 7,337 (US$ 142.76) per month to meet their minimum basic food needs (PSA, 2018b). The study revealed that the average income mostly crabbing households in Tigbauan Municipality was way below the indicated poverty threshold. Though some of the crabbers engaged in other livelihood opportunities, such as carpentry, fish vending, and construction works, the marginal contribution from these earnings to the household income was still inadequate to supplement the basic and other needs of the family. Other sources of income are needed for the crabbing families to live a decent life. The reason for this state of living, according to them, was the decline in catch of BSC and other target crabs as well as the increasing number of crabbers in the area, which contributed to the lowering of catch per crabber. Another aspect was their educational attainment. The lack of formal education beyond high school severely limits alternative employment opportunities (Rhodes et al., 2001). This might be one factor that limits the crabbers from having a high-paid job. However, as per observation, the crabber's children were all sent to schools aiming to have a better future.

Crabbing Gears and Practices

The crabbing villages in the Municipality of Tigbauan, Iloilo, used two types of fishing gears in their crabbing operations namely bottom-set gillnet or crab entangling net and crab pots. Ingles (2004) pointed out in his paper that these two are the major crabbing gears used in the Philippines. Namocon, Atabayan, Baguingin, and Barroc used crab pots, while Parara Norte was the only village that used bottom-set gillnet or crab entangling net. The use of this other type of gear in Parara Norte was due to the influence and assistance given by the government through projects implemented by the Bureau of Fisheries and Aquatic Resources (BFAR), an agency of the Philippine government under the Department of Agriculture responsible for the development, improvement, management, and conservation of the Philippines' fisheries and aquatic resources. This particular village was the only beneficiary of the implemented project done in their locality.

Table 2. Socio-demographic profile of crabbers in Tigbauan, Iloilo, central Philippines

Respondents (24)	Crabbers' Count	Percentage Composition (%)
Gender		
Male	24	100
Years of Residence		
16 – 20 years	2	8.3
21 – 25 years	1	4.2
26 – 30 years	2	8.3
>30 years	19	79.2
Total	24	100
Religion		
Roman Catholic	24	100
Civil Status		
Married	24	100
Household Members		
1 – 5	10	41.7
6 – 10	14	58.3
Total	24	100
Educational Attainment		
Elementary level	2	8.3
Elementary graduate	4	16.7
High School level	3	12.5
High School graduate	8	33.3
College level	1	4.2
College graduate	1	4.2
Vocational course	5	20.8
Total	24	100
Average monthly income (PHP)		
1,000 – 5,000	13	54.2
6,000 – 10,000	7	29.2
11,000 – 15,000	2	8.3
26,000 – 30, 000	1	4.2
31,000 – 35, 000	1	4.2
Total	24	100
Years of engagement in crab fishing		
1 – 5	5	20.8
6 – 10	3	12.5
11 – 15	4	16.7
16 – 20	1	4.2
21 – 25	2	8.3
26 – 30	2	8.3
More than 30	7	29.2
Total	24	100
Owned a lot		
Yes	12	50
No	12	50
Total	24	100
Tenure of house		
Informal settlers	12	100
Member of BFARMC		
Yes	22	91.7
No	2	8.3

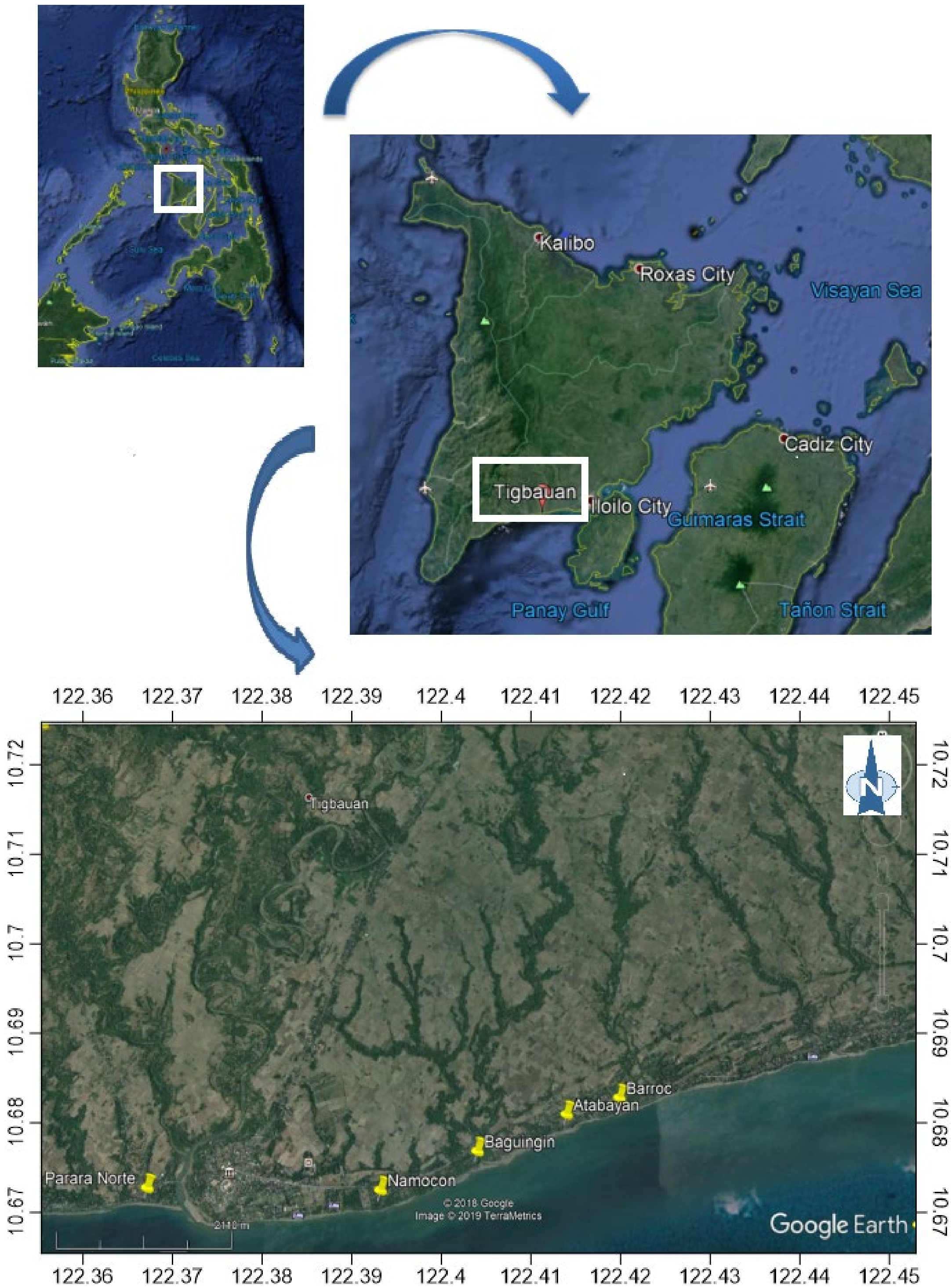

Figure 1. Map of the study site

Crab pot

A crab pot is a type of gear designed for catching commercially important crab species. The gear is locally known as *bubo pangasag, panggal* or *timing* (Mesa et al., 2018) and is more favored among crabbers due to its cheaper cost, and it catches less by-catch (Romero, 2009). The gear used in Tigbauan, Iloilo, has a frustum shape with different sizes or dimensions. Crab pots used in shallow waters are larger than the ones used in deeper waters.

Crab pots used in shallow areas are usually made of ground wire for the frame, which is also served as sinker of the gear, wrapped around with polyethylene net and have an opening made from plastic hexagonal mesh-shaped wire having a dimension of height is 15.24 cm; diameter at the top is 35.56 cm; diameter at the bottom is 43.18 - 48.26 cm. The door with a rectangular-shaped, where the catch is collected, which is 17.78 × 17.78 cm, has an opening, which serves as an entry point of target species, measuring 20.32 cm (Figure 2). Another entry point design is circular, having a diameter of 11.43 cm, and is made of green-colored plastic hexagonal mesh-shaped wire (Figure 3). Crab pot cost per unit is PHP100.00 (US$1.90). The only distinct design used in the shallow area is the collapsible crab pot design that was donated to a fisherman from Sweden. It is made of a color black coated steel bar frame with black netting and with a rectangular shape having the following dimension: length is 59.70 cm; width is 17 cm; height is 15.20; and with an opening of 20.30 cm. Net mesh size is number 14. The openings or found on both sides of the gear (Figure 4).

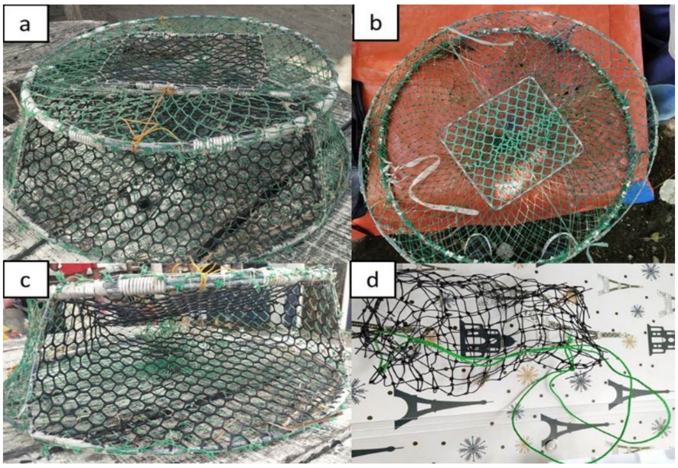

Figure 2. Crab pot used in shallow waters in Namocon, Tigbauan, Iloilo, central Philippines, showing the (a) side view of the crab pot, (b) top view with the door, (c) opening or the entry point of the crab and (d) mesh net where the bait is being put and tied inside the crab pot

Figure 3. Crab pot used in shallow waters in Namocon, Tigbauan, Iloilo, central Philippines with circular-shaped entry point

In deeper waters, crab pots are made of bamboo wrapped with polyethylene green-colored net and monofilament nylon. According to crabbers, white nets have been used recently because it is easier to clean and more visible for crabs to see the bait inside the trap. Crab pots' sizes are almost uniform in the three villages with a height of 11.43 to 15.24 cm, a top diameter of 30.48 cm, and bottom diameter of 40.64 cm, an opening of 10.16 cm, which is made of green colored plastic chicken wire and a mesh size of 2.5 cm (Figure 5). Sinkers used are made of cement attached inside directly built in the flooring of the pot for the stability of the gear when deployed underwater. The said gear can accommodate 10 pieces of small size crabs or 3 - 4 pieces of large sizes. Crab pot typically costs PHP70.00 (US$ 1.36).

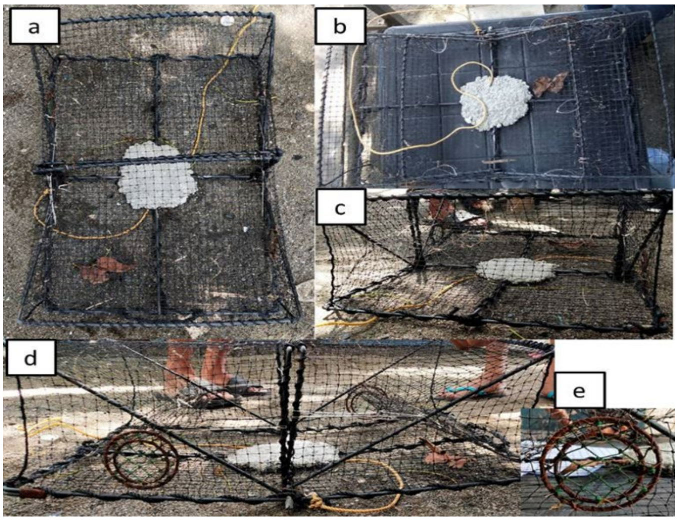

Figure 4. Collapsible crab trap designed from Sweden used in shallow waters in Namocon, Tigbauan, Iloilo, central Philippines showing the (a) top view of crab trap, (b) collapsed form, (c) opening or the entry point of the crab, (d) side view of the crab trap and (e) escape point of small size crabs

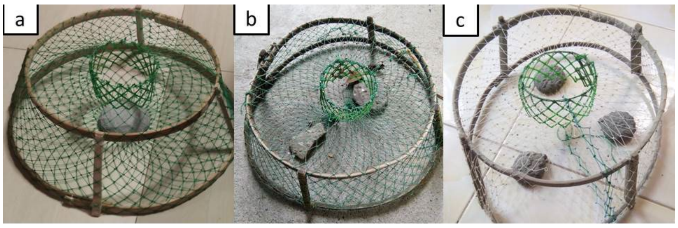

Figure 5. Crab pots used in deep water operation in Tigbauan, Iloilo, central Philippines for crab fishing operation; (a) crab pot use in Barroc, (b) crab pot use in Baguingin and (c) crab pot use in Atabayan

Bottom-set gillnet/Crab entangling net

The bottom set gillnet or the crab entangling net is a type of gillnet anchored and fixed on the seafloor. The gear is locally known as *palubog, palugdang or pukot* (Mesa et al., 2018). The net used in the village of Parara Norte is made of monofilament polyamide (PA), having a length of 600 m. Mesh sizes vary every 200 m. Monofilament PA size number 4 (approx. 85 mm) or size number 5 (78.5 mm) on first 200 m, size number 4.5 (approx. 85 mm) on second 200 m (middle part), and size number 3 (130 mm) on the last 200 m. The design was made by local fishers to maximize the chance of catching more crabs. The mainline was made of a monofilament PA size number 6 attached with rubber floaters. The footrope was also made of monofilament PA size number 6 with leads that serve as sinkers (Figure 6).

Figure 6. Bottom-set gill net used in Parara, Tigbauan, Iloilo, central Philippines for crab fishing; (a) monofilament net with number 5 mesh size, (b) nylon used for mainline and footrope, (c) floaters attached at the mainline and (d) lead sinkers attached at the footrope

Crabbing Practices/Operation

The crabbing operation varied in each area according to its fishing gear used. For the bottom-set gill net or crab entangling net, the setting is usually done in the morning at around 5 o'clock, and hauling is done on the next morning at around 5-8 o'clock. Soaking time is approximately 24 hours. The said operation is also similar to that is shown in the study of Romero (2009) and Mesa et al. (2018). However, Romero (2009) added that due to the eagerness of crabbers to gain more income, they would be setting out their operation further using bigger boats for 2-3 days at sea to catch more BSCs. Motorized banca, approximately 3 gross tons (GT) (Figure 7), is used by two persons for their crabbing operation. On the other hand, crabbers in the Western Visayan Sea use bigger boats with the size of 10-30 ft long powered by 8, 10-16 HP engines (Romero, 2009). Peak season in using bottom-set gill net is during southwest monsoon or *habagat*, *i.e.,* June to October of the year, while lean season happens every November to December.

The bottom-set gillnet or crab entangling net has a total stretch length of 600 m. During operation, nets are deployed at the desired depth. Mesh size varies every 200 m-length of the net with a hanging ratio of 0.6. The distance from the surface of the water to the mainline is approximately 9-10 m. The net used has a mesh size count of 50 knots down. Lead sinkers tied in the rope line will serve as the weights to keep the net at a desired level of depth (Figure 8).

Crab pot fishing operation in the four villages varied as well. Namocon village operates in shallow waters, while villages such

Figure 7. Motorized banca used by two crabbers in Parara Norte, Tigbauan, Iloilo, central Philippines

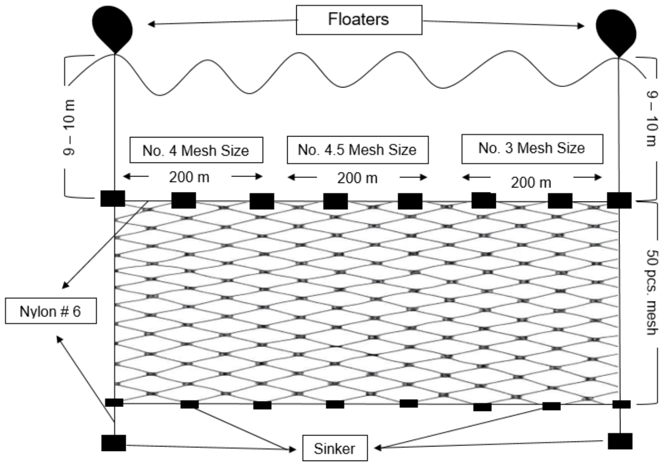

Figure 8. Lay-out of crab fishing operation using bottom-set gillnet or crab entangling net in Parara Norte, Tigbauan, Iloilo, central Philippines

as Baguingin, Atabayan, and Barroc operate in deeper waters. The setting of crab pots in shallow waters is done by carrying the pots to the fishing area and setting it manually by crabbers at around 4 - 5 p.m. Hauling is done on the next day at around 4 - 5 a.m. also. Soaking time is approximately 12 hours. The distance of operation from the shoreline is approximately 10 m away. Ten to twenty-five crab pots are used per operation. Bait used by the crabbers is small fishes, usually *Leiognathus* sp., and/or chicken head. The chicken head is preferred by Namocon village because it is cheaper and readily available in the local market. However, the silvery color of leiognathids believed to glint in the water, therefore, assists in the attraction of organisms into the gear (Picoy-Gonzales & Monteclaro, Unpublished data). The baits are also placed in a perforated container to prevent them from being eaten by scavengers (Bjordal, 2002). Peak season occurs when the water is turbid and when the typhoons are frequent, which is usually every June to September of the year. On the other hand, the lean season is every October to March of the year when the water is clear. And at this time, crabbers do not operate at all to avoid losses in terms of exerting effort. Instead, they engage in other possible alternative income whenever opportunities let them. The shallow water crabbing operation is shown in Figure 9.

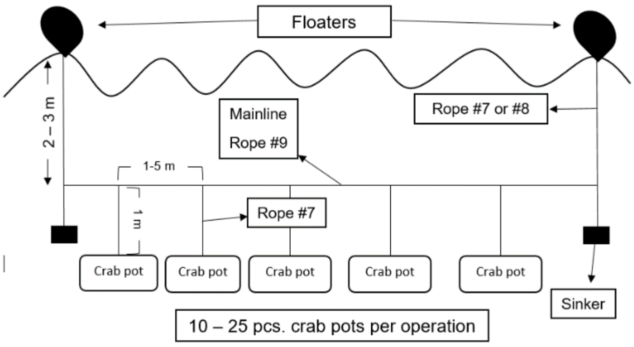

Figure 9. Lay-out of crab fishing operation using crab pots in Namocon, Tigbauan, Iloilo, central Philippines

Figure 10. Motorized crabbing boat used in fishing operation in the deeper waters of Tigbauan, Iloilo, central Philippines

In deeper waters, the operation is done using a larger motorized banca (Figure 10) boarded by two persons. The setting is usually done at 5 - 9 a.m., and hauling is done on the next day at around 4 - 5 a.m. Soaking time is approximately 24 hours. Crab pots used range from 150 - 300 units per operation. Bait used in every operation is small fishes with low market value (*e.g.,* leiognathids), same with Namocon village, which was purchased from the local market. Formerly, crabbers used frogs as bait because it is cheaper and more efficient. However, buyers opted to buy the crabs that are fed with frogs due to the perception of the consumers that they were also indirectly eating the frog as well. The peak season for the deep-water operation is usually October to June, while the lean season is somewhere from July to September. Crabbers can attain a catch up to 10 kilograms of crabs every peak season. However, during the lean season, they could just acquire a half kilo or no catch at all.

Crab pot fishers, which are engaged in deeper water, typically operate near the Guimaras area with depths ranging from 15-20 fathoms. However, when water is clear, the operation is usually done 100 - 200 m away from the shoreline. The set-up of fishing gear underwater is shown in the figure below. The length of the rope from the floaters, which flags are used with varieties of colors such as red, orange, and black to be easily seen and identified, is around 5-36 m depending on the fisherman's depth preference to operate. The mainline used is PE rope number 10 or 12, where crab pots are tied individually. The distance between each crab pot is 3-7 m. Each crab pot is still tied with another rope, which is PE rope number 6 or 8, to the mainline with a distance of 0.5 - 1.5 m. The whole set-up is weighted by 3 steel bar sinkers. Crabbers usually used 200 - 300 crab pot units per operation. The more the crab pots are used, the lesser its distance away from each pot (Figure 11).

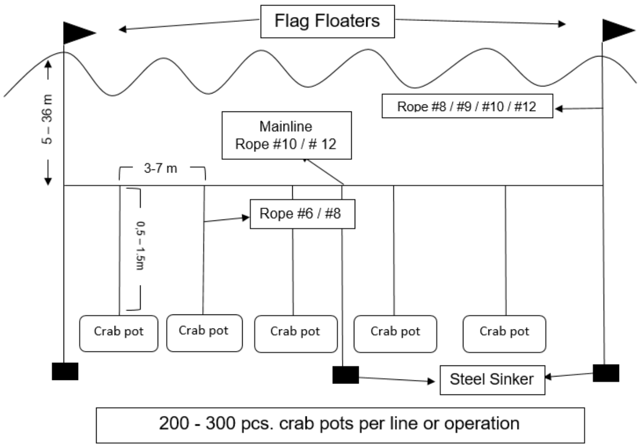

Figure 11. Lay-out of crab fishing operation using crab pots in Barroc, Atbayan and Baguingin, Tigbauan, Iloilo, central Philippines

Crab fishing in the Municipality of Tigbauan, Iloilo, is done every day or even twice a day if it is peak season. No catch limit was implemented per day per fisher, and the only ordinance is no catching of gravid females and crablets of less than 27.94 cm. This is stated in the Municipal Ordinance No. 2014 - 10, "Environment Code of the Municipality of Tigbauan" under Chapter 10, Article J of Section 2.5, which penalized the violators a fine of PHP 2,500.00 (US$ 48.67) or will be imprisoned for not less than 5 days or both depending on the discretion of the court.

BSC is normally traded alive starting from the landing until it reaches the end consumers, for it commands a higher price. Crabbers usually let the crab recover by submerging them underwater before selling to the market. However, dead individuals are also sold at the same price provided that those of good quality or fat. Species caught are only sold at the local market with a price of PHP280.00 (US$ 5.45) if it is in small sizes and can reach up to PHP 420.00 (US$ 8.18) at larger sizes. Species usually caught in every operation are *Portunus pelagicus (kasag), Charybdis feriata (kurusan), Portunus sanguinolentus (pintokan),* and *Scylla serrata (alimango*). By-catch includes flatfish, lobster, and stingrays.

Awareness of Regulatory Measures Regarding Crab Fishery Management

The only ordinance of the Municipality of Tigbauan in Iloilo with regards to crab fishing is the Municipal Ordinance No. 2014-10, "Environment Code of the Municipality of Tigbauan." Chapter 10, Article J, Section 2.5 prohibits taking, catching, selling, processing, or transporting gravid crabs and crablets, mud crabs and/or blue crabs with a carapace less than 11.00 cm. This coincides with the size of BSC at first maturity, which is 10.56 cm for females and 9.64 cm for males. Violations on the said provisions will be penalized of PPH 2,500.00 (US$ 48.67) or will be imprisoned for not less than 5 days or both depending on the discretion of the court. According to the survey, all crabbers are fully aware of the ordinance (Table 3), and the information was disseminated properly by the LGU. However, the lack of enforcement or strict implementation is still the constraint of this ordinance. The release of the species is still at the fishers' discretion and guilt.

Table 3. Ordinance awareness of crabbers in Tigbauan, Iloilo, central Philippines

Respondents (24)	Crabbers' Count	Percentage Composition (%)
Yes	24	100
No	0	0
Total	24	100

Problems and Concerns

The major problems and concerns among crabbers are the trawling and/or dragging activities in the area. The municipality has already formulated an ordinance to ban the use of the said destructive gear. However, operations are continuously rampant. Whenever trawling is operated after crab pots are being deployed, these fishing gears are being swept away and nowhere to be found. This could lead to loss of income to crabbers and add up to the cost of making new ones. Concerns were brought to LGU but accordingly, no actions were made and done. Crabber's feedback is that implementation of the ordinance is too weak in the area. No "bantay dagat" (sea guard) were assigned to each station to guard against this illegal activity. The second concern is poaching. Other fishers tend to harvest the crabs that were caught by their crabbing gears. Next is the use of drift gillnets or locally known *palutaw,* which could also drag and damage the mainline of the crab pots, resulting in the loss of the gears. Typhoons are also considered a problem since they could no longer operate during this period due to strong winds and waves. Therefore, it is too risky for crabbers to operate in deeper areas. At this point, they do not have an income to support their families, especially those who are only relying on crab fishing as their major source of income.

Suggestions to Improve the Crab Fishery in Tigbauan, Iloilo

As observed during the conduct of the survey, crabbing is done on a regular basis (except for bad weather) without considering the reproduction period of the BSC. Seldom, catching of gravid females is prevalent. Even though there is an ordinance of prohibition as mentioned above and all crabbers are well-informed, they tend to be tempted because there are times when the catch is scarce. In their minds also, when they return the caught gravid BSC, another crabber will fish it anyway. The probability of survival as well might be low due to stress during the catching and handling process. A study must be conducted on the reproductive biology of BSC taking into account its biology in the area, which could be a basis for the formulation of an effective management plan or of having a closed season to prevent overfishing of the stock. Crabbers tend to harvest one piece of crab for an overnight soaking time which could be a red flag of stock depletion. Stock enhancement might be a solution to address this phenomenon. Alternative livelihood should be given to crabbers to also reduce the fishing pressure. They could probably shift to having a business rather than crab fishing. Providing them with alternative livelihoods is a means of reducing fishing efforts in the area. The government support is necessary and should be appropriate. Fishing gears given to the fishers should fit on what is being used. A preliminary survey should be done before distributing supports so that efficient help is extended appropriately and efficiently.

Conclusion

Five villages were involved in crab fishing in the Municipality of Tigbauan, central Philippines. Almost all crabbers and their family in the area remained poor because of their lack of formal education, which limited them from engaging in other types of livelihood that would increase their income. The majority of the income, which came from the BSC fishery, was barely enough to support the basic needs of the family. In terms of fishing gears, crab pots and crab entangling net or bottom-set gillnet were used by crabbers, and no modifications were found. Fishing operation varied per type of gear; however, species caught were similar. The daily operation may contribute to the decline of the catch of BSC. Thus, catching of gravid female BSC is inevitable. Hence, this paper suggests a comprehensive stock assessment program may be conducted to determine the dynamics of the population and the status of the habitat in the area. A review on reproductive biology in the area must be given attention to establish the appropriate time. In terms of giving alternative livelihood, a consultation with the end-users must be done to give the appropriate help. Lastly, the ordinance must be strictly implemented to help crab fishery in the municipality in the long run, and sustainability will not be compromised.

Compliance With Ethical Standards

Authors' Contributions

MLTF and ABT designed the study. MLTF wrote the initial draft, and ABT edited it. Both authors read and approved the final version of the article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

BFAR (Bureau of Fisheries and Aquatic Resources). (2013). The blue swimming crab management plan. Retrieved from http://www.bfar.da.gov.ph

Bjordal, A. (2002). The use of technical measures in responsible fisheries: Regulation of fishing gear. In Cochrane, K. L. (Ed.), *A fishery manager's guidebook*: *Management measures and their application* (pp. 21-48). FAO Fisheries Technical Paper. No. 424. FAO.

Camacho, A. S., & Aypa, S. M. (2001). Research needs and data on production of portunid crabs in the Philippines. *Asian Fisheries Science*, *14*(2), 243-245.

de la Cruz, M. T., de la Cruz, J. O., Ruizo, E. K. C., & Tan, I. L. (2018). The blue swimming crab fishers and fishing practices in Leyte and Samar, Philippines. *The Philippine Journal of Fisheries*, *25*(2), 1-15. https://doi.org/10.31398/tpjf/25.2.2018-0001

De la Cruz, M. T., De la Cruz, J. O., Tan, I. L., & Ruizo, E. K. (2015). The blue swimming crab (*Portunus pelagicus*) fishery of Eastern Visayas, Philippines. *Philippine Journal of Natural Sciences*, *20*(1), 25-45.

Del Norte-Campos, A. G. C., Villarta, K. A., Panes, J. B., Declarador, M. (2004). Catch and catch rates of the blue swimming crab (*Portunus pelagicus* L.) in various fishing frounds in Panay Island. *UPV Journal of Natural Sciences, 9*(1), 79-86.

Gadhavi, M. K., Kardani, H. K., Rajal, P., Prajapati, P. C., & Vachhrajani, K. D. (2013). Impact of trawl fish ban on artisanal brachyuran crab fishery in and around Sikka, Gulf of Kutch, Gujarat, India. *Research Journal of Animal, Veterinary and Fishery Sciences*, *1*(1), 22-27.

Germano, B. P., & Melgo, J. L. F. (2003). Population, reproductive and fishery biology of the blue crab, *Portunus pelagicus*, in Leyte and Samar, and management implications. *UPV Journal of Natural Sciences, 8*, 63-82.

Ingles, J. A. (2004). Status of the blue crab fisheries in the Philippines. In DA-BFAR (Department of Agriculture- Bureau of Fisheries and Aquatic Resources), *In Turbulent seas: The status of Philippine marine fisheries* (pp. 47-52). Coastal Resource management Project, Department of Agriculture- Bureau of Fisheries and Aquatic Resources. Philippines.

Mesa, S. V., Bayate, D. E. E., & Guanco, M. R. (2018). Blue Swimming Crab Stock Assessment in the Western Visayan Sea. *The Philippine Journal of Fisheries*, *25*(1), 77-94.

Picoy-Gonzales, R. M., & Monteclaro, H. M. (Unpublished Data). Value chain analysis of blue swimming crab *Portunus pelagicus* in Tigbauan, Iloilo, Philippines. 63 p.

PSA (Philippine Statistics Authority). (2018a). January – March 2018 Fisheries Situation Report. Philippine Statistics Authority.

PSA (Philippine Statistics Authority). (2018b). Official poverty statistics of the Philippines for first semester 2018. Retrieved from https://psa.gov.ph

Romero, F. G. (2009). Population structure of the blue crabs, *Portunus pelagicus* (L.) in the Visayan Sea: Implications to fisheries management [Ph.D. Thesis. University of the Philippines].

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RESEARCH ARTICLE

Growth promoter, immunostimulant and antioxidant for rainbow trout (*Oncorhynchus mykiss*): Terebinth (*Pistacia terebinthus*) extract

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ABSTRACT

In this study, the effects of *Pistacia terebinthus* (PT) fruit extract supplemented diet on growth performance, haematology, digestive and antioxidant enzyme activities, and non-specific immune responses were evaluated in juvenile rainbow trout (*Onchoryhnchus mykiss*). The fish were fed diets containing three doses of *Pistacia terebinthus* extract (0.1, 0.5 and 1% of diet) and a control diet without extract for 63 days. Final weight, weight gain and specific growth rate were significantly improved in all the treated groups. In addition, feed conversion ratio was significantly reduced in all PT diet fed groups. Pepsin and lipase activities were significantly increased in all the treated groups. Trypsin was significantly improved in PT 0.1% and PT 1% groups. Amylase was significantly increased in PT 0.5 and 1% groups. In haematological assays, red blood cell, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration values were not changed among all experimental groups. Superoxide dismutase, catalase, glutathione peroxidase and glucose-6-phosphate dehydrogenase activities were significantly improved in all the treatment groups. However, catalase activity decreased in PT 0.5% group at the end of 63 days. In addition, hepatic and white muscle lipid peroxidation activities were significant decreased in all the treated groups compared to the control. Non-specific immune parameters, such as nitroblue tetrazolium reduction, myeloperoxidase and lysozyme activities were increased in all the treated fish groups. These results indicated that extract of *P. terebinthus* can be used to improve fish health in aquaculture.

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Introduction

Fish is one of the important protein sources in human diet. Over the last two decades, aquaculture sector has developed rapidly and expanded to many countries (FAO, 2018). However, this development causes various economic issues such as disease risk, use of expensive feed, wrong feeding strategy or formulation and others (Farahi et al., 2010; Syahidah et al., 2015; Erguig et al., 2015). Nutritional management is an important key factor for prevention of diseases and successful production in aquaculture (Farahi et al., 2010; Bilen & Bilen, 2013).

Inadequate nutritional factors are known to cause various diseases and poor health condition in fish (Waagbø, 1994). Adequate nutrients in diet directly affect to improve fish health and prevent disease. Therefore, strategies for nutritional management have critical role in the success of semi-intensive and intensive aquaculture (Zoral et al., 2018). Also, the increase of technological developments gave an opportunity to increase stocking densities in aquaculture facilities (Bilen et al., 2013), which in turns could also negatively affect fish health.

Fish farmers usually use antibiotics or some chemotherapeutics for the prevention and control of diseases (Boran et al., 2013; Cabello et al., 2016; Uney et al., 2021). However, antibiotics and chemotherapeutics may pose residual and toxic effects on organs in fish and human (Erguig et al., 2015; Syahidah et al., 2015). In addition, the use of antibiotics and chemotherapeutic agents for controlling diseases can pollute the aquatic environment (Biswas et al., 2010; Syahidah et al., 2015).

Since the past decade, application of medicinal plants and their bioactive compounds are being widely used to prevent diseases and maintain fish health in aquaculture (Hoseinifar et al., 2019; Bilen et al., 2020; Hoseinifar et al., 2020a; Hoseinifar et al., 2020b; Terzi et al., 2021; Yılmaz et al., 2022). Bioactive compounds of these herbs have various beneficial effects to animal (Güroy et al., 2022; Sönmez et al., 2019; Sönmez et al., 2021). For example, several reports demonstrated that medicinal plants have antimicrobial, anti-antioxidant, appetite stimulating, digestibility ameliorative, growth promoting, sperm quality improving and immunostimulant properties (Ranjbar et al., 2010; Banaee et al., 2011; Takaoka et al., 2011; Vaseeharan & Thaya, 2014; Sönmez et al., 2018; Sönmez et al, 2019; Karga et al., 2020).

Pistacia terebinthus L. (terebinth), belonging to the family Anacardiaceae, is a plant that widely grows in the Mediterranean region countries, such as Morocco, Portugal, Greece, Turkey, Syria and Lebanon (Topçu et al., 2007). It was reported that terebinth can be used to treat bronchitis, wounds, burns and stomach disorders (Topçu et al., 2007; Cakilcioglu & Turkoglu, 2010; Gogus et al., 2011). In addition, pharmacological studies have revealed that terebinth has antimicrobial, antifungal, antioxidant, anti-inflammatory, diuretic and antitussive properties (Topçu et al., 2007; Özcan et al., 2009). There are no reports on the application of this herb for improvement of growth and health of rainbow trout (*Onchoryhnchus mykiss*).

In this context, an experiment was conducted to investigate the efficacy of terebinth supplemented diet on rainbow trout health. We examined various parameters (growth performance, digestive and antioxidant enzyme activities, haematological profile, and immune responses) which are directly related to aquatic animal health.

Material and Methods

Source of fish and acclimatization

Rainbow trout juveniles (15.77±0.13 g) were purchased from a commercial fish farm in Kastamonu, Turkey. All experiments were performed at the indoor laboratory of Kastamonu University, Fisheries Faculty, Turkey. The fish were acclimatized for 2 weeks before commencement of the experiment. Water parameters (7.77-9.29 mg/L of dissolved oxygen, 6.5-7.5 pH, 12°C water temperature) were checked daily. Photoperiod was maintained at 12 h light: 12 h dark. During acclimation, the fish were fed *ad libitum* with a commercial diet twice a day.

Preparation of terebinth extract

Ripe fruits of terebinth were collected according to the procedure described by Pakravan et al. (2012) with a slight modification. The ripe fruits were ground in a mechanical grinder to a fine powder. Fifty gram of fruits and 1 L of 40% methanol (Sigma-Aldrich, St. Louis, MO, USA) were blended in a laboratory blender. The mixture was kept at room temperature for 3 days with daily shaking. After 3 days, fruit extract was filtered through a filter paper (Whatman filter No 1). Then, the filtrate was collected and evaporated in a rotary evaporator at 55-65°C to remove alcohol from the fruit extract. Final product (crude) was dissolved in distilled water and kept in a flask at 4°C until use for experiment. The final product was sprayed on fish diet (Commercial fish feed (Özpekler MoyFeed, 2 mm, Protein 48%, Fat 17%) at three selected doses, 0.1, 0.5 and 1% terebinth extract diet (Taee et al., 2017). All the experimental diets were kept in plastic zipped packs and stored at -20°C until use. Diet composition is shown in Table 1.

Table 1. Composition of the experimental diets

Constituents	Concentration (%)			
	Control	PT	PT	PT
	0	0.1%	0.5%	1%
Crude protein	46	46	46	46
Total lipid	14	14	14	14
Moisture	11	11	11	11
Ash	10	10	10	10
Phosphorus	1.2	1.2	1.2	1.2
Fiber	3	3	3	3
PT fruit extract	0	0.5	0.1	1

Note: PT: *Pistacia terebinthus*, PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract

Oral treatment trials and sampling

The fish were randomly divided into four main groups that were fed with 3 experimental diets, 0.1, 0.5 and 1% *Pistacia terebinthus* and a control diet without extract. Forty fish were distributed in each tank (500 L, 1 m diameter) in triplicate. All the groups were fed *ad-libitum* for 63 days twice in a day.

During oral administration experiment, fish (3 fishes from each experimental tanks) were randomly selected and were anesthetised (0.01 mg/L of fenoxyethanol) for sampling at 21, 42 and 63 days. Blood was collected from caudal vein puncture with heparinized syringes. The blood was transferred to EDTA tubes for analysis of haematological and non-specific immune parameters. The blood was centrifuged at 10000 rpm for 15 min at 4°C. And then, plasma was separated and stored at -80°C.

Stomach and anterior part of intestine were collected, and stored in plastic tubes at -80°C at the end of experiment. Liver and white muscle samples were collected at 21, 42 and 63 days and stored in liquid nitrogen at -80°C until antioxidant enzyme and lipid peroxidation analyses were performed.

All animal experiments were conducted according to the relevant international guidelines. Study protocols were approved in advance by the local ethics committee for animal research studies at the Kastamonu University (KUHADYEK-07.03.2016-2016.10).

Growth performance

Weight of fish was measured at the beginning and at the end of study. Each fish was individually weighed. Growth performance was calculated as follows:

$$\text{Weight gain (WG,\%)} = \left[100 \times \frac{\text{Final fish weight-Initial fish weight}}{\text{Initial fish weight}}\right] \quad (1)$$

$$\text{Specific growth rate (SGR,\%/day)} = \left[100 \times \frac{\ln(\text{final fish weight}) - \ln(\text{initial fish weight})}{\text{Experimental days}}\right] \quad (2)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}} \quad (3)$$

$$\text{Survival rate (SR,\%)} = \left[\frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100\right] \quad (4)$$

Digestive enzyme activities

The stomach and anterior part of intestine tissues (0.1 g) were homogenized in 1 mL cold double distilled water using ISOLAB homogenizer (Germany). And then, samples were centrifuged at 9000 rpm for 20 min at 4°C. The supernatant was separated and stored at –80°C until digestive enzyme activity assay. Protein contents of stomach and intestine supernatants were determined according to Bradford (1976).

Pepsin activity in stomach supernatant was determined as described by Anson (1938). Trypsin activity in intestinal supernatant was determined using benzoyl-dl-arginine-p-nitroanilide following Erlanger et al. (1961). Lipase activity in the intestinal supernatant was determined by hydrolysis of 4-nitrophenyl myristate (Gawlicka et al., 2000). Amylase activity in the intestinal supernatant was determined by starch hydrolysis (Worthington, 1991).

Heamatological analysis

Red blood cell (RBC, $\times 10^6/mm^3$) was measured as described by Blaxhall & Daisley (1973). Hemoglobin (Hb, g dL^{-1}) was determined according to Drabkin & Austin (1932). Hematocrit (Hct, %) was determined as described by Billett (1990). Red blood indices such as mean cell volume (MCV, fL), mean cell hemoglobin (MCH, pg) and mean cell hemoglobin concentration (MCHC, %) were calculated according to White et al. (2006).

$$\text{Mean cell volume (MCV, } \mu m^3) = \left[\frac{\text{Hct (\%)}}{\text{RBC}(\times 10^6 \mu L)} \times 10\right] \quad (5)$$

$$\text{Mean cell hemoglobin (MCH, pg)} = \left[\frac{\text{Hb (g } dL^{-1})}{\text{RBC}(\times 10^6 \mu L)} \times 10\right] \quad (6)$$

$$\text{Mean cell hemoglobin concentration (MCHC, \%)} = \left[\frac{\text{Hb (g } dL^{-1})}{\text{Hct(\%)}}\right] \quad (7)$$

Antioxidant activity

Liver (0.1 g) and white muscle (0.1 g) samples were washed with physiological saline. Samples were dried on a filter paper and then homogenized separately on ice with 1 mL of phosphate buffer. After that, the samples were centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was then separated and stored at -80°C until use for oxidative stress activity assay. The protein contents of liver supernatants were determined similarly as described above (Bradford, 1976).

Superoxide dismutase (SOD) in liver tissue was determined with using Sigma-Aldrich kit (Cat. No. 19160). Catalase (CAT), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PDH) and lipid peroxidation (LPO) were determined using Cayman chemical kits, item No. 707002, 703102, 700300 and 10009055, respectively.

Non-specific immune parameters

Nitroblue tetrezolium (NBT) reduction activity was determined using fresh blood (Siwicki et al., 1994). Myeloperoxidase (MPO) and lysozyme activity (LA) assays were determined using plasma according to (Ellis, 1990; Sahoo et al., 2005) with some modifications.

Statistical analysis

The data were expressed as mean ± SEM (standard error of means) for all parameters tested. Differences between treatments for various parameters were determined by one-way analysis of variance (ANOVA) followed by Tukey tests at 5% level of significance ($p<0.05$) using SPSS (16.0) program.

Results

Growth performance

All diet administered groups demonstrated that final weight and weight gain were significantly higher ($p<0.05$) than in control (Table 2). In addition, rate of WG and SGR were significantly increased compared to the control (Table 2). FCR in all the treatment groups significantly decreased (Table 2). During the feeding experimental period, no mortality was observed in all the groups.

Digestive enzyme activities

Pepsin and amylase activity in 0.1, 0.5 and 1% PT diet fed groups significantly increased ($p<0.05$) in a dose-dependent manner (Table 3). Trypsin activity significantly increased in 0.1 and 1% PT diet fed groups (Table 3). In addition, lipase activity significantly increased in all the treatments (Table 3).

Haematological analysis

RBC, Hct, MCV, MCH and MCHC values did not vary among treatment groups and control (Table 4). Only 0.5% PT diet fed fish had significantly increased Hb level compared to that of other diet fed groups and control (Table 4).

Antioxidant activity

After 21 days of feeding, SOD activity increased significantly ($p<0.05$) in 0.1 and 0.5% PT diet administered groups (Fig. 1). Additionally, after 42 days, all treatment groups showed a significantly higher SOD activity compared to that of control. SOD activity was higher in 0.1 and 1% PT diet fed groups at the end of experiment (Figure 1). Throughout the feeding period, CAT activity in 0.1% PT diet fed group was significantly higher than that in control (Figure 2). In addition, other treatment groups displayed higher CAT activity compared to control at different sampling days (Figure 2). GPx activity in all PT diet fed groups increased in a dose-dependent manner after 21 and 63 days (Figure 3). We observed that G6PDH activity in all the treatment groups increased at 21 and 42 days (Figure 4). Only 0.5% PT diet fed fish did not display any significant result compared to control at the end of study (Figure 4). On the other hand, LPO activity significantly decreased in all treatment groups compared to that of control (Figure 5).

Table 2. Growth performance parameters and survival of rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Growth Parameters					
	Initial weight (g)	*Final weight (g)*	*Weight gain (%)*	*FCR*	*SGR (%)*	*Survival (%)*
Control	15.58±0.06	42.12± 0.35[b]	170.37± 2.70[b]	1.18± 0.00[a]	1.58±0.02[b]	100
PT0.1%	15.38± 0.14	51.01± 0.36[a]	232.5.1± 2.67[a]	1.02± 0.00[b]	1.91± 0.01[a]	100
PT0.5%	15.41± 0.12	50.79± 0.26[a]	229.82± 3.87[a]	1.03± 0.00[b]	1.89± 0.02[a]	100
PT1%	15.27± 0.18	51.54± 0.40[a]	236.66± 4.95[a]	1.04± 0.00[b]	1.92± 0.02[a]	100

Note: Each value is mean ± SE. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. FCR, feed conversion ratio; SGR, specific growth rate. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Table 3. Digestive enzyme activities of rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Digestive Enzyme Activities			
	Pepsin *U/mg protein*	*Trypsin* *U/mg protein*	*Lipase* *U/mg protein*	*Amylase* *U/mg protein*
Control	47.95 ± 0.69[d]	8.29 ± 0.16[b]	0.15 ± 0.11[b]	0.51 ± 0.01[c]
PT0.1%	67.40 ± 0.65[b]	12.62 ± 0.57[a]	0.23 ± 0.11[a]	0.56 ± 0.03[c]
PT0.5%	75.45 ± 0.88[b]	8.31 ± 0.48[b]	0.19 ± 0.02[a]	0.68 ± 0.03[a]
PT1%	81.68 ± 0.81[a]	11.45 ± 0.55[a]	0.20 ± 0.01[a]	0.59 ± 0.02[b]

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences (p<0.05) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Table 4. Haematological parameters of rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Haematological Parameters					
	RBC *($x10^6/mm^3$)*	*Hb* *(g/dl)*	*Hct* *(%)*	*MCV* *(μm^3)*	*MCH* *(pg)*	*MCHC* *(%)*
Control	2.14 ± 0.03	8.47±0.44[b]	23.00 ± 0.57	107.43 ± 3.73	43.79 ±0.34	441.66 ± 1.20
PT0.1%	1.94± 0.10	9.63± 0.43[b]	22.43 ± 0.80	112.37± 1.52	46.49 ±0.58	440.33 ± 0.66
PT0.5%	2.31 ± 0.06	9.90± 0.36[a]	23.37 ± 0.46	101.53 ± 2.49	43.45 ±1.48	440.66 ± 1.20
PT1%	2.07 ± 0.05	9.37± 0.35[b]	22.07 ± 0.76	104.83 ± 3.27	45.13 ±0.78	442.00 ± 1.52

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences (p<0.05) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract. RBC, Red blood cell; Hb, haemoglobin; Hct, haematocrit; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration.

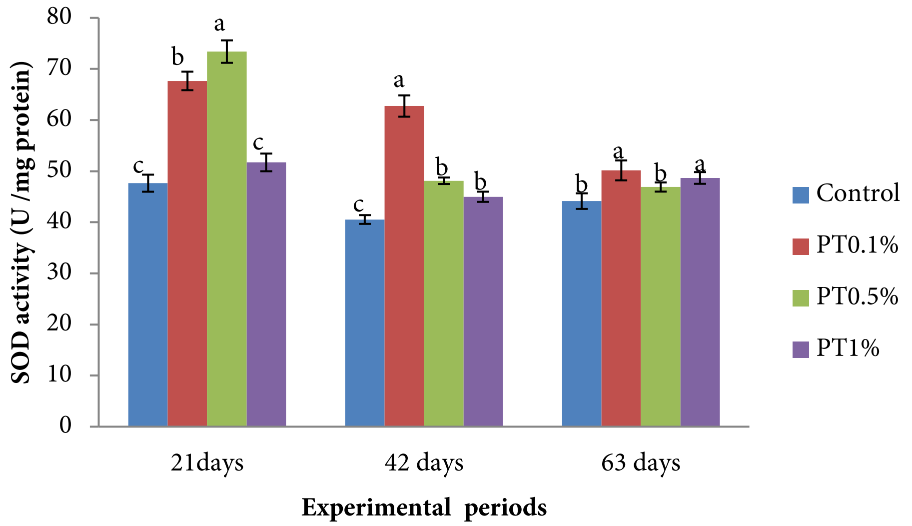

Figure 1. Superoxide dismutase (SOD) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences (*p<0.05*) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

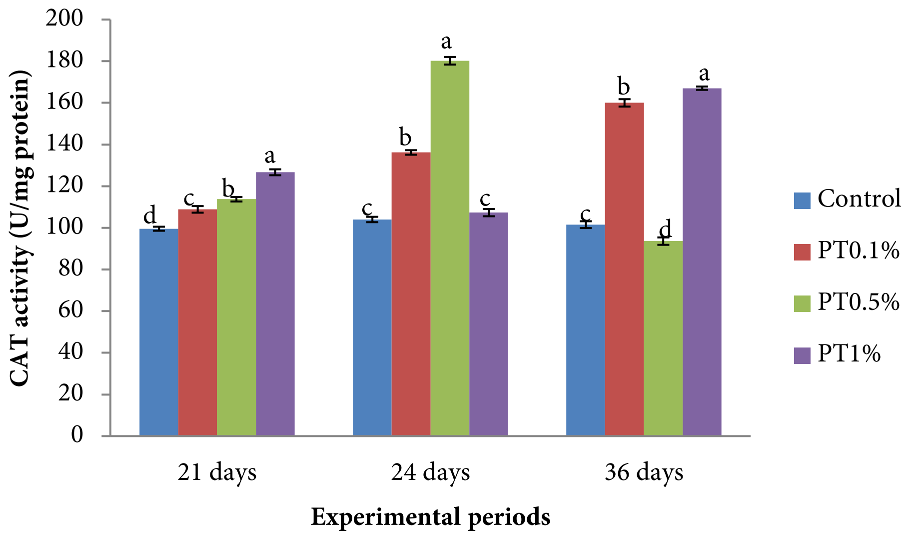

Figure 2. Catalase (CAT) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences (*p<0.05*) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

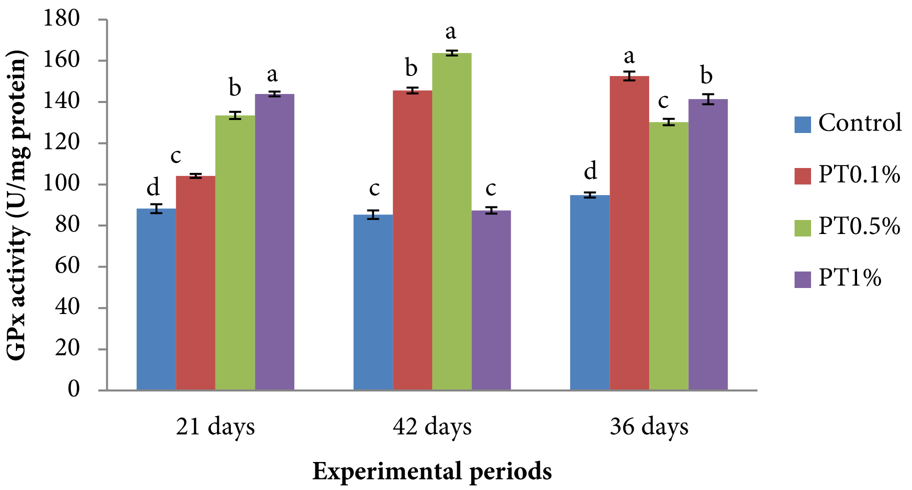

Figure 3. Glutathione peroxidase (GPx) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences (*p<0.05*) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Non-specific immune parameters

During the experiment, MPO activity significantly increased in fish of all treatment groups (Table 5). In addition, we observed that NBT level in all PT diet fed treatments increased at the end of 21, 42 and 63 days (Table 6). Increasing LA activity was observed on different sampling day in treatment groups (Table 7).

Discussion

In the present study, dietary administration of PT extract supplemented feed on the growth parameters (FW, WG and SGR) indicated beneficial effects in rainbow trout. Moreover, FCR decreased significantly in all PT extract treated fish groups. Major bio-active compounds of terebinth, such as vitamins, minerals, polysaccharide, alkaloids, fatty acids and flavonoids could affect the growth rate. Previous studies reported that these compounds stimulated appetite which caused enhanced feed consumption, digestion and absorption of nutrients (Zahran et al., 2014; Bilen et al., 2020; Elbesthi et al., 2020). In contrast, Bilen & Bilen (2012) found no differences on growth performance in rainbow trout fed with tetra and laurel powder. The performance of digestive system in fish and other animals directly depends on enzyme (pepsin, trypsin, amylase and lipase) activities (Furne et al., 2012). Pepsin is one of the most important acid proteases secreted in stomach. This enzyme is responsible for the digestion of dietary proteins to polypeptides such as phenylalanine, tyrosine and other aromatic amino acids (Darias et al., 2007). In this study, pepsin activity significantly increased in all PT diet fed groups. This increase may be due to protein contents in terebinth extract, which could stimulate pepsin activity in the stomach. Previous studies reported that pepsin activity was significantly increased in rainbow trout fed garlic (*Allium sativum*), lupin (*Lupinus perennis*), stinging nettle (*Urtica dioica*) and mango (*Mangifera indica*) incorporated diets (Nya & Austin, 2011; Awad et al., 2012). Trypsin enzyme, one of the alkaline proteases, is secreted in intestine lumen. It completes protein digestion process and hydrolysis of amino acids when chyme arrives at the intestine (Darias et al., 2007; Napora-Rutkowski et al., 2009). The current study revealed that PT extract at 0.1 and 1% of diet caused a significant improvement in the intestinal trypsin activity compared to that of the control and 0.5% PT extract treated group. Probably, terebinth compounds accelerated trypsin activity in the intestine. Ester bonds among the fatty acids and glycerol are dissolved by lipase enzyme which is secreted from pancreas to be discharged into intestine lumen (Ojha et al., 2014). An enhanced trypsin activity was also observed in carp fed with *Chenopodium album* (Amhamed et al., 2018) and *Apium graveolens* extracts (Mohamed et al., 2018). Similar to trypsin activity, lipase activity increased in all the treated fish groups compared to the control. This is probably due to terebinth compounds that stimulated lipase activity. The amylase enzyme is mainly responsible for degradation of carbohydrates and secreted in pancreas and finally discharged into intestine lumen. It is responsible for the hydrolysis of glycosidic bonds among sugar residues in large carbohydrates. It digests carbohydrates into glucose (Ojha et al., 2014). Amylase activity increased in all the treated groups. The increase in amylase activity might have happened due to carbohydrate content in fruit extract of PT or may be due to augmentation for proliferation and maintenance of intestinal microflora which can produce enzymes that may improve digestibility and absorption of feed ingredients (Aly & Mohamed 2010; Citarasu, 2010; Bulfon et al., 2015). These findings agree with the previous studies which indicated that medicinal herbal extracts stimulated digestion with enhanced bile acid concentration and induced the pancreas for increased secretion of digestive enzymes in fish (Bhosale et al., 2010).

Table 5. Myeloperoxidase activity (OD 450 nm) in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Experimental Period		
	21 days	*42 days*	*63 days*
Control	108.67 ± 1.34^{d}	116.80 ± 2.02^{d}	110.89 ± 1.75^{c}
PT0.1%	128.81 ± 2.92^{c}	156.39 ± 2.26^{a}	111.12 ± 3.42^{c}
PT0.5%	151.22 ± 2.67^{a}	139.65 ± 3.00^{b}	114.64 ± 2.57^{b}
PT1%	137.11 ± 1.45^{b}	126.59 ± 1.76^{c}	122.87 ± 3.61^{a}

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Table 6. Nitroblue tetrezolium activity (mg ml^{-1}) in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days.

Treatment Groups	Experimental Period		
	21 days	*42 days*	*63 days*
Control	1.38 ± 0.02^{c}	2.45 ± 0.04^{c}	2.33 ± 0.05^{c}
PT0.1%	1.63 ± 0.05^{b}	2.81 ± 0.05^{a}	2.76 ± 0.06^{a}
PT0.5%	1.67 ± 0.04^{b}	2.58 ± 0.04^{b}	2.51 ± 0.06^{b}
PT1%	2.59 ± 0.02^{a}	2.58 ± 0.01^{b}	2.74 ± 0.02^{a}

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Table 7. Lysozyme activity (U ml-1) in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	**Experimental Period**		
	21 days	***42 days***	***63 days***
Control	0.45 ± 0.00[b]	0.02 ± 0.00[b]	0.02 ± 0.00[c]
PT0.1%	0.47 ± 0.03[b]	0.05 ± 0.01[b]	0.02 ± 0.01[c]
PT0.5%	0.61 ± 0.02[a]	0.03 ± 0.00[b]	0.03 ± 0.01[b]
PT1%	0.37 ± 0.03[c]	0.27 ± 0.02[a]	0.04 ± 0.01[a]

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences (p<0.05) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

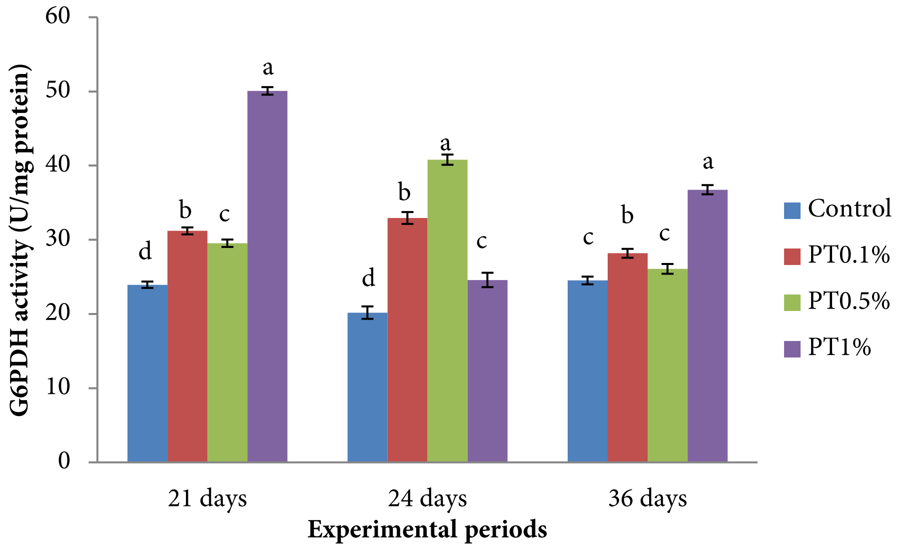

Figure 4. Glucose-6-phosphate dehydrogenase (G6PDH) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences (*p<0.05*) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

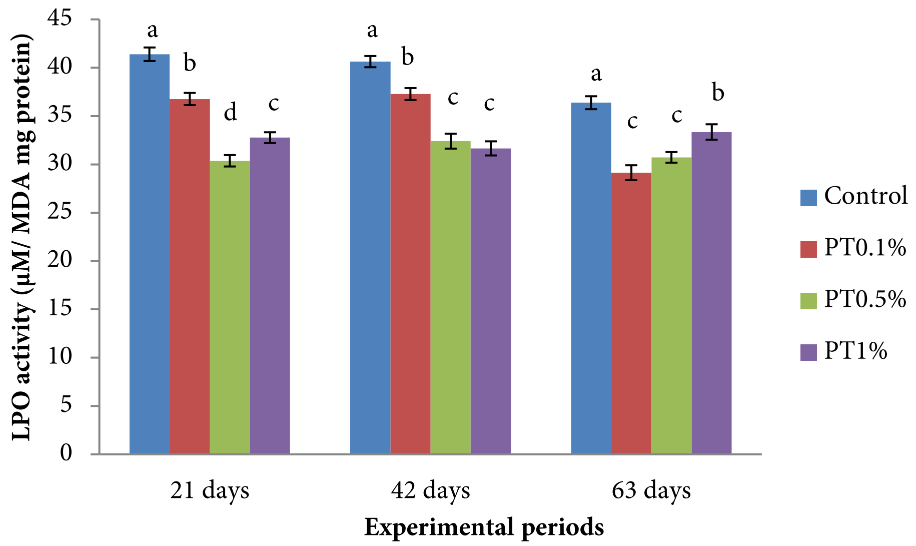

Figure 5. Lipid peroxidation (LPO) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences (*p<0.05*) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

The present study showed that RBC, Hb, Hct, MCV, MCH and MCHC values did not vary among treatments and control. This result established that supplementation of terebinth extract at 0.1, 0.5 and 1% of feed does not exert any adverse effect on haematological parameters in rainbow trout.

SOD and CAT are responsible for removal of superoxide anions (Vutukuru et al., 2006). In the present study, SOD and CAT activities increased in all the treatment groups at all sampling times, except in 0.5% PT extract administered group after 63 days. This increase in both hepatic SOD and CAT activities was probably due to the antioxidant property of fruit extract which could further stimulate antioxidant defence mechanism through enhancing the activity of endogenous antioxidant enzymes or stimulating the synthesis of hepatic SOD and CAT to prevent generation of excessive free radicals (O_2^- and H_2O_2) during normal cell metabolism and respiratory burst activity (Topçu et al., 2007; Yilmaz et al., 2010). GPx is one of the important enzymes involved in cellular antioxidant enzymatic system. In addition, the GPx activity is an indicator of cell damage (Uner et al., 2006; Liang et al., 2017). In the present study, the hepatic GPx activity was significantly increased in all the experimental groups throughout the experimental period. The antioxidant property of terebinth extract might have stimulated GPx activity. Ellagic acid is one of the main phenolic compounds of *Pistasia spesices* (Mehenni et al., 2016). In line with the present finding, Mişe Yonar et al. (2014) observed that GPx activity in rainbow trout increased significantly during feeding with ellagic acid. Similar result of increased GPx activity was also reported in rainbow trout fed with thyme (*Thymus vulgaris*), sage (*Salvia officinalis*) and mint (*Mentha spicata*) extracts (Sönmez et al., 2015). G6PDH is considered as a secondary antioxidant enzyme (Rayeni, 2016). It cannot neutralise free radicals directly, however, it has a supporting role to other endogenous antioxidants (Carocho & Ferreira, 2013). The current study demonstrated that G6PDH activity increased in all the treatment groups after 21 and 42 days. Only in fish fed with 0.5% PT diet, no significant enhancement was observed after 63 days. Sönmez et al. (2015) reported similar results of elevated G6PDH activity in rainbow trout fed with thyme, sage and mint extracts. Furthermore, clove (*Syzygium aromaticum*) and cardamom (*Elettaria cardamomum*) containing diets increased the activity of G6PDH in rohu fish (*Labeo rohita*) (Asimi & Sahu, 2016). LPO was significantly reduced in all the experimental fish groups. This result may be attributed to free radical scavenging property of terebinth extract which is associated to the bioactive compounds such as polyphenols, flavonoids and vitamins. Mişe Yonar et al. (2014) suggested ellagic acid as a polyphenolic compound (Mehenni et al., 2016) that caused a significant decrease in LPO in liver, kidney and spleen tissues in rainbow trout. In addition, LPO activity decreased in rainbow trout and silver catfish (*Rhamdia quelen*) fed with the medicinal herb, garlic which contains flavonoid compounds (Mohebbi et al., 2012; Pês et al., 2016).

Superoxide anions are important antimicrobial effectors. Thus, the respiratory burst activity of phagocytes has been used frequently as an indicator of non-specific immunity in fish (Sahoo et al., 2005). NBT reduction activity improved in fish of the all experimental groups. This effect may be due to a strong immunostimulant property of this fruit extract which contains bioactive compounds, such as vitamins, oils, polyphenolic and flavonoid compounds (Topçu et al., 2007; Karga et al., 2020). In line with our results, *Aloe vera* extracts caused enhanced respiratory burst activity in rainbow trout fry (Haghighi et al., 2014). MPO is an enzyme secreted by macrophages and neutrophils of fishes. It utilizes halide and hydrogen peroxidase to kill bacteria (Hampton et al., 1996). The MPO can act as an indicator of phagocyte and neutrophil activities, which are used as an indicator of non-specific immune response (Johnston, 1978). MPO activity increased in all treatment fish groups. Similar result was observed in Mozambique tilapia (*Oreochromis mossambicus*) fed a diet supplemented with a medicinal herb, guduchi (*Tinospora cordifolia*) leaves (Alexander et al., 2010) and rainbow trout fed with cherry stem extract (Amoush et al., 2021). LA is an important element in non-specific defence mechanism (Evelyn, 2002). Lysozyme is secreted by lysosomes present in neutrophils (Uribe et al., 2011). In our study, LA increased on different sampling days in treatment groups. Some previous studies indicated that medicinal herbs and their bioactive compounds have positive effects on LA activity in rainbow trout (Salem et al., 2021; Lakwani et al., 2022). Haghighi et al. (2014) reported that LA activity increased significantly in rainbow trout fry fed with 1% of *Aloe vera* extract.

Conclusion

In conclusion, our results indicated that terebinth extract supplemented diet has positive effect on growth, digestive enzyme activities, antioxidant activities and non-specific immune response in rainbow trout. It is also considered that this type of medicinal plants can be used in aquaculture sector as a therapeutic and immunostimulant supplement material.

Compliance With Ethical Standards

Authors' Contributions

GAM: During experimental period fish car, laboratory studies and writing.

SB: Experimental design preparing, laboratory studies, statistical analyses and writing.

KG: The plant collection, preparing of the extraction and GS-MS analyses.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

All animal experiments were conducted according to the relevant international guidelines. Study protocols were approved in advance by the local ethics committee for animal research studies at the Kastamonu University (KUHADYEK-07.03.2016-2016.10).

References

Alexander, C. P., Kirubakaran, C. J. W., & Michael, R. D. (2010). Water soluble fraction of *Tinospora cordifolia* leaves enhanced the non-specific immune mechanisms and disease resistance in *Oreochromis mossambicus*. *Fish & Shellfish Immunology*, *29*, 765-772. https://doi.org/10.1016/j.fsi.2010.07.003

Aly, S. M., & Mohamed, M. F. (2010). *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*). *Journal of Animal Physiology and Animal Nutrition*, *94*(5), e31-e39. https://doi.org/10.1111/j.1439-0396.2009.00971.x

Amhamed, I. D., Mohamed, G. A., Almabrok, A. A., Altıef, S., Abdalsalam, T., & Bilen, S. (2018). Efficacy of dietary *Chenopodium album* extract on some health parameters, digestive enzymes and growth performance in juvenile *Cyprinus carpio*. *Alinteri Journal of Agriculture Sciences*, *33*(2), 165-176. https://doi.org/10.28955/alinterizbd.412455

Amoush, O. A., Bilen, S., Sönmez, A. Y., & Elp, M. (2021). Antioxidant and immunostimulant responses in rainbow trout (*Oncorhynchus mykiss*) fed with cherry stem extract. *Aquaculture Research*, *28*(3), 869-899. https://doi.org/10.1007/s10499-019-00501-3

Anson, M. L. (1938). The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. *The Journal of General Physiology*, *22*(1), 79-89. https://doi.org/10.1085/jgp.22.1.79

Asimi, O., & Sahu, N. (2016). Effect of antioxidant rich spices, clove and cardamom extracts on the metabolic enzyme activity of *Labeo rohita*. *Journal of Fisheries & Livestock Production*, *4*, 157. https://doi.org/10.4172/2332-2608.1000157

Awad, E., Austin, B., & Lyndon, A. (2012). Effect of dietary supplements on digestive enzymes and growth performance of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of American Science*, *8*(12), 858-864.

Banaee, M., Sureda, A., Mirvaghefi, A. R., Rafei, G. R. (2011). Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, *37*, 885-896. https://doi.org/10.1007/s10695-011-9486-z

Bhosale, S. V., Bhilave, M., & Nadaf, S. (2010). Formulation of fish feed using ingredients from plant sources. *Research Journal of Agricultural Sciences*, *1*, 284-287.

Bilen, S., & Bilen, A. M. (2012). Growth promoting effect of tetra (*Cotinus coggygria*) and laurel (*Laurus nobilis*) on rainbow trout (*Oncorhynchus mykiss*). *Alinteri Zirai Bilimler Dergisi*, *22*, 26-33.

Bilen, S., & Bilen, A. M. (2013). Effects of different protein sources on growth performance and food consumption of goldfish, *Carassius auratus*. *Iranian Journal of Fisheries Sciences*, *12*(3), 717-722.

Bilen, S., Ali, G. A. M., Amhamed, I. D., & Almabrok, A. A. (2021). Modulatory effects of laurel-leaf cistus (*Cistus laurifolius*) ethanolic extract on innate immune responses and disease resistance in common carp (*Cyprinus carpio*). *Fish & Shellfish Immunology*, *116*, 98-106. https://doi.org/10.1016/j.fsi.2021.07.001

Bilen, S., Ispir, S., Kenanoglu, O. N., Taştan, Y., Güney, K., & Terzi, E. (2020). Effects of Greek juniper (*Juniperus excelsa*) extract on immune responses and disease resistance against *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*, *44*(6), 729-738. https://doi.org/10.1111/jfd.13293

Bilen, S., Karga, M. K., Altunoglu, Y. Ç., Ferhat, U., & Biswas, G. (2020). Immune responses and growth performance of the aqueous methanolic extract of *Malva sylvestris* in *Oncorhynchus mykiss*. *Marine Science and Technology Bulletin*, *9*(2), 159-167. https://doi.org/10.33714/masteb.746951

Bilen, S., Kızak, V., & Bilen, A. M. (2013). Floating fish farm unit (3FU): Is it an appropriate method for salmonid production? *Marine Science and Technology Bulletin*, *2*, 9-13.

Billett, H. H. (1990). Hemoglobin and hematocrit. In Walker, H. K., Hall, W. D., & Hurst, J. W. (Eds.), *Clinical Methods: The History, Physical, and Laboratory Examinations* (3rd edition). Butterworths.

Biswas, A., Kondaiah, N., Anjaneyulu, A., & Mandal, P. (2010). Food safety concerns of pesticides, veterinary drug residues and mycotoxins in meat and meat products Asian Journal of Animal Sciences, *4*(2), 46-55. https://doi.org/10.3923/ajas.2010.46.55

Blaxhall, P.C., & Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, *5*(6), 771-781 https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Boran, H., Terzi, E., Altinok, I., Capkin, E., & Bascinar, N. (2013). Bacterial diseases of cultured Mediterranean horse mackerel (*Trachurus mediterraneus*) in sea cages. *Aquaculture*, *396-399*, 8-13. https://doi.org/10.1016/j.aquaculture.2013.02.025

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Bulfon, C., Volpatti, D., & Galeotti, M (2015). Current research on the use of plant-derived products in farmed fish. *Aquaculture Research*, *46*(3), 513-551. https://doi.org/10.1111/are.12238

Cabello, F. C., Godfrey, H. P., Buschmann, A. H., & Dolz, H. J. (2016). Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. *The Lancet Infectious Disease*, *16*(7), e127-e133. https://doi.org/10.1016/s1473-3099(16)00100-6

Cakilcioglu, U., & Turkoglu, I. (2010). An ethnobotanical survey of medicinal plants in Sivrice (Elazig-Turkey). *Journal of Ethnopharmacology*, *132*(1), 165-175 https://doi.org/10.1016/j.jep.2010.08.017

Carocho, M., & Ferreira, I. C. (2013). A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, *51*, 15-25. https://doi.org/10.1016/j.fct.2012.09.021

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, *18*, 403-414. https://doi.org/10.1007/s10499-009-9253-7

Darias, M. J., Murray, H. M., Gallant, J. W., Douglas, S. E., Yufera, M., & Martinez-Rodriguez, G. (2007). Ontogeny of pepsinogen and gastric proton pump expression in red porgy (*Pagrus pagrus*): Determination of stomach functionality. *Aquaculture*, *270*, 369-378 https://doi.org/10.1016/j.aquaculture.2007.04.045

Drabkin, D. L., & Austin, J. H. (1932). Spectrophotometric studies I. Spectrophotometric constants for common hemoglobin derivatives in human, dog, and rabbit blood. *Journal of Biological Chemistry*, *98*, 719-733.

Elbesthi, R. T. A., Özdemir, K. Y., Taştan, Y., Bilen, S., Sönmez, A. Y. (2020). Effects of ribwort plantain (*Plantago lanceolata*) extract on blood parameters, immune response, antioxidant enzyme activities, and growth performance in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, *46*(4), 1295-1307. https://doi.org/10.1007/s10695-020-00790-z

Ellis, A. E. (1990). Lysozyme Assays. In Stolen, J. S., Fletcher, T. C., Anderson, D. P., Roberson, B. S., & Van Muiswinkel, W. B. (Eds.), *Techniques in Fish Immunology* (pp. 101-103). SOS Publications.

Erguig, M., Yahyaoui, A., Fekhaoui, M., & Dakki, M. (2015). The use of garlic in aquaculture. *European Journal of Biotechnology and Bioscience*, *3*(8), 28-33.

Erlanger, B. F., Kokowsky, N., & Cohen, W. (1961). The preparation and properties of two new chromogenic substrates of trypsin. *Archives of Biochemistry and Biophysics*, *95*(2), 271-278. https://doi.org/10.1016/0003-9861(61)90145-X

Evelyn, T. P. T. (2002). Finfish immunology and its use in preventing infectious diseases in cultured finfish. In Lavilla-Pitogo, C. R., & Cruz-Lacierda, E. R. (Eds.), *Diseases in Asian Aquaculture IV: Proceedings of the Fourth Symposium on Diseases in Asian Aquaculture*, Philippines, pp. 303-324.

FAO. (2018). *The state of world fisheries and aquaculture*. Rome: FAO.

Farahi, A., Kasiri, M., Sudagar, M., Iraei, M. S., Shahkolaei, M. D. (2010). Effect of garlic (*Allium sativum*) on growth factors, some hematological parameters and body compositions in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, Aquarium, Conservation & Legislation*, *3*(4), 317-323.

Furne, M., Morales, A. E., Trenzado, C. E., Garcia-Gallego, M., Hidalgo, M. C., Domezain, A., & Rus, A. S. (2012). The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. *Journal of Comparative Physiology B*, *182*, 63-76. https://doi.org/10.1007/s00360-011-0596-9

Gawlicka, A., Parent, B., Horn, M. H., Ross, N., Opstad, I., Torrissen, O. J. (2000). Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): Indication of readiness for first feeding. *Aquaculture*, *184*, 303-314. https://doi.org/10.1016/S0044-8486(99)00322-1

Gogus, F., Ozel, M. Z., Kocak, D., Hamilton, J. F., & Lewis, A. C. (2011). Analysis of roasted and unroasted *Pistacia terebinthus* volatiles using direct thermal desorption-GCxGC-TOF/MS. *Food Chemistry*, *129*, 1258-1264. https://doi.org/10.1016/j.foodchem.2011.05.003

Güroy, B., Güroy, D., Bilen, S., Kenanoğlu, O. N., Şahin, I., Terzi, E., Karadal, O., & Mantoğlu, S. (2022). Effect of dietary spirulina *(Arthrospira platensis*) on the growth performance, immune-related gene expression and resistance to *Vibrio anguillarum* in European seabass (*Dicentrarchus labrax*). *Aquaculture Research*, *53*(6), 2263-2274. https://doi.org/10.1111/are.15745

Haghighi, M., Sharif Rohani, M., Samadi, M., Tavoli, M., Eslami, M., & Yusefi, R. (2014). Study of effects *Aloe vera* extract supplemented feed on hematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*). *International Journal of Advanced Biological and Biomedical Research*, *2*(6), 2143-2154.

Hampton, M. B., Kettle, A. J., & Winterbourn, C. C. (1996). Involvement of superoxide and myeloperoxidase in oxygen-dependent killing of *Staphylococcus aureus* by neutrophils. *Infection and Immunity*, *64*(9), 3512-3517.

Hoseinifar, S. H., Shakouri, M., Van Doan, H., Shafiei, S., Yousefi, M., Raeisi, M., Yousefi, S., Harikrishnan, R., & Reverter, M. (2020a). Dietary supplementation of lemon verbena (*Aloysia citrodora*) improved immunity, immune-related genes expression and antioxidant enzymes in rainbow trout (*Oncorrhyncus mykiss*). *Fish & Shellfish Immunology*, *99*, 379-385. https://doi.org/10.1016/j.fsi.2020.02.006

Hoseinifar, S. H., Sohrabi, A., Paknejad, H., Jafari, V., Paolucci, M., & Van Doan, H. (2019). Enrichment of common carp (*Cyprinus carpio*) fingerlings diet with *Psidium guajava*: The effects on cutaneous mucosal and serum immune parameters and immune related genes expression. *Fish & Shellfish Immunology*, *86*, 688-694. https://doi.org/10.1016/j.fsi.2018.12.001

Hoseinifar, S. H., Sun, Y.-Z., Zhou, Z., Van Doan, H., Davies, S. J., & Harikrishnan, R. (2020b). Boosting immune function and disease bio-control through environment-friendly and sustainable approaches in finfish aquaculture: herbal therapy scenarios. Reviews in Fisheries Science & Aquaculture, 28(3), 303-321

Johnston, J. R. (1978). Oxygen metabolism and the microbicidal activity of macrophages. *Federation Proceedings*, *37*(13), 2759-2764.

Karga, M., Kenanoğlu, O. N., & Bilen, S. (2020). Investigation of antibacterial activity of two different medicinal plants extracts against fish pathogens. *Journal of Agricultural Production*, *1*(1), 5-7. https://doi.org/10.29329/agripro.2020.341.2

Lakwani, M. A., Kenanoğlu, O. N., Taştan, Y., & Bilen, S. (2022). Effects of black mustard (*Brassica nigra*) seed oil on growth performance, digestive enzyme activities and immune responses in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, *53*(1), 300-313. https://doi.org/10.1111/are.15577

Liang, X. -P., Li, Y., Hou, Y. -M., Qiu, H., & Zhou, Q. -C. (2017). Effect of dietary vitamin C on the growth performance, antioxidant ability and innate immunity of juvenile yellow catfish (*Pelteobagrus fulvidraco* Richardson). *Aquaculture Research*, *48*(1), 149-160. https://doi.org/10.1111/are.12869

Mehenni, C., Atmani-Kilani, D., Dumarçay, S., Perrin, D., Gérardin, P., & Atmani, D. (2016). Hepatoprotective and antidiabetic effects of *Pistacia lentiscus* leaf and fruit extracts. *Journal of Food and Drug Analysis*, *24*(3), 653-669. https://doi.org/10.1016/j.jfda.2016.03.002

Mişe Yonar, S., Yonar, M., Yöntürk, Y., & Pala, A. (2014). Effect of ellagic acid on some haematological, immunological and antioxidant parameters of rainbow trout (*Oncorhynchus mykiss*). *Journal of Animal Physiology and Animal Nutrition*, *98*(5), 936-941. https://doi.org/10.1111/jpn.12162

Mohamed, G. A., Amhamed, I. D., Almabrok, A. A., Barka, A. B. A., Bilen, S., & Elbishti, R. T. (2018). Effect of celery (*Apium graveolens*) extract on the growth, haematology, immune response and digestive enzyme activity of common carp (*Cyprinus carpio*). *Marine Science and Technology Bulletin*, *7*, 51-59.

Mohebbi, A., Nematollahi, A., Dorcheh, E. E., & Asad, F. G. (2012). Influence of dietary garlic (*Allium sativum*) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, *43*(8), 1184-1193. https://doi.org/10.1111/j.1365-2109.2011.02922.x

Napora-Rutkowski, L., Kamaszewsk, M., Bielawski, W., Ostaszewska, T., & Wegner, A. (2009). Effects of starter diets on pancreatic enzyme activity in juvenile sterlet (*Acipenser ruthenus*). *The Israeli Journal of Aquaculture – Bamidgeh*, *61*(2), 143-150.

Nya, E. J., & Austin, B. (2011). Dietary modulation of digestive enzymes by the administration of feed additives to rainbow trout, *Oncorhynchus mykiss* Walbaum. *Aquaculture Nutrition*, *17*(2), e459-e466. https://doi.org/10.1111/j.1365-2095.2010.00782.x

Ojha, M., Chadha, N., Saini, V., Damroy, S., Chandraprakash, S. P., & Sawant, P. (2014). Effect of ethanolic extract of Mucuna pruriens on growth, metabolism and immunity of *Labeo rohita* (Hamilton, 1822) fingerlings. *International Journal of Fauna and Biological Studies*, *1*(5), 01-09.

Özcan, M. M., Tzakou, O., & Couladis, M. (2009). Essential oil composition of the turpentine tree (*Pistacia terebinthus* L.) fruits growing wild in Turkey. *Food Chemistry*, *114*(1), 282-285. https://doi.org/10.1016/j.foodchem.2008.08.094

Pakravan, S., Hajimoradloo, A., & Ghorbani, R. (2012). Effect of dietary willow herb, *Epilobium hirsutum* extract on growth performance, body composition, haematological parameters and *Aeromonas hydrophila* challenge on common carp, *Cyprinus carpio*. *Aquaculture Research*, *43*(6), 861-869. https://doi.org/10.1111/j.1365-2109.2011.02901.x

Pês, T. S., Saccol, E. M., Ourique, G. M., Londero, É. P., Gressler, L. T., Finamor, I. A., Rotili, D. A., Golombieski, J. I., Glanzner, W. G., Llesuy, S. F., Gonçalves, P. B., Radünz Neto, J., Baldisserotto, B., & Pavanato, M. A. (2016). Effect of diets enriched with rutin on blood parameters, oxidative biomarkers and pituitary hormone expression in silver catfish (*Rhamdia quelen*). *Fish Physiology and Biochemistry*, *42*(1), 321-333. https://doi.org/10.1007/s10695-015-0140-z

Ranjbar, M., Ghorbanpoor, M., Peyghan, R., Mesbah, M. (2010). Effects of dietary *Aloe vera* on some specific and nonspecific immunity in the common carp (*Cyprinus carpio*). *Iranian Journal of Veterinary Research*, *4*(3), 189-195. https://dx.doi.org/10.22059/ijvm.2010.21352

Rayeni, M. (2016). Influence of plant antioxidant on the shelf-life of seafood. *International Journal of Agriculture and Biosciences*, *5*(4), 221-226.

Sahoo, P., Kumari, J., Mishra, B. (2005). Non-specific immune responses in juveniles of Indian major carps. *Journal of Applied Ichthyology*, *21*(2), 151-155. https://doi.org/10.1111/j.1439-0426.2004.00606.x

Salem, M. O. A., Salem, T. A., Yürüten Özdemir, K., Sönmez, A. Y., Bilen, S., & Güney, K. (2021). Antioxidant enzyme activities and immune responses in rainbow trout (*Onchorhynchus mykiss*) juveniles fed diets supplemented with dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) extracts. *Fish Physiology and Biochemistry*, *47*(4), 1053-1062. https://doi.org/10.1007/s10695-021-00962-5

Siwicki, A. K., Anderson, D. P., & Rumsey, G. L. (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology*, *41*(1-2), 125-139. https://doi.org/10.1016/0165-2427(94)90062-0

Sönmez, A. Y., Bilen S., Alak G., Hisar O., Yanık T., Biswas G. (2015). Growth performance and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets supplemented with sage, mint and thyme oils. *Fish Physiology and Biochemistry*, *41*(1), 165-175. https://doi.org/10.1007/s10695-014-0014-9

Sönmez, A. Y., Bilen, S., Taştan, Y., Serag, K. J. B., Toring, C. C., Romero, J. B., Kenanoğlu, O. N., & Terzi, E. (2021). Oral administration of *Sargassum polycystum* extracts stimulates immune response and increases survival against *Aeromonas hydrophila* infection in *Oncorhynchus mykiss*. *Fish & Shellfish Immunology*, *117*, 291-298. https://doi.org/10.1016/j.fsi.2021.08.020

Sonmez, A. Y., Ozdemir, R. C., Bilen, S., & Kadak, A. E. (2019). Effect of ginseng root (*Araliaceae* sp.) extracts on sperm quality parameters and reproductive performance in rainbow trout (*Oncorhynchus mykiss*). *The Israeli Journal of Aquaculture-Bamidgeh*, *71*, IJA_71.2019.1570.

Sönmez, A., Özdemir, R. C., Bilen, S., & Yürüten Özdemir, K. (2018). Cyclopamine induced expression of immune-related genes in rainbow trout (*Oncorhynchus mykiss*) head kidney leukocytes. *The Israeli Journal of Aquaculture- Bamidgeh*, *70*, IJA_70.2018.1560.

Syahidah, A., Saad, C. R., Daud, H. M., & Abdelhadi, Y. M. (2015). Status and potential of herbal applications in aquaculture: A review. *Iranian Journal of Fisheries Sciences*, *14*(1), 27-44.

Taee, H. M., Hajimoradloo, A., Hoseinifar, S. H., Ahmadvand, H. (2017). The effects of dietary myrtle (*Myrtus communis* L.) supplementations on growth performance and some innate immune responses in rainbow trout (*Oncorhynchus mykiss*). *International Journal of Aquatic Biology*, 5(4), 252-259. https://doi.org/10.22034/ijab.v5i4.331

Takaoka, O., Ji, S. -C., Ishimaru, K., Lee, S., Jeong, G. S., Ito, J., Biswas, A. (2011). Effect of rotifer enrichment with herbal extracts on growth and resistance of red sea bream, *Pagrus major* (Temminck & Schlegel) larvae against *Vibrio anguillarum*. *Aquaculture Research, 42*(12), 1824-1829. https://doi.org/10.1111/j.1365-2109.2010.02783.x

Terzi, E., Kucukkosker, B., Bilen, S., Kenanoglu, O. N., Corum, O., Özbek, M., & Parug, S. S. (2021). A novel herbal immunostimulant for rainbow trout (*Oncorhynchus mykiss*) against *Yersinia ruckeri*. *Fish & Shellfish Immunology*, *110*, 55-66. https://doi.org/10.1016/j.fsi.2020.12.019

Topçu, G., Ay, M., Bilici, A., Sarikürkcü, C., Öztürk, M., & Ulubelen, A. (2007). A new flavone from antioxidant extracts of *Pistacia terebinthus*. *Food Chemistry*, *103*(3), 816-822. https://doi.org/10.1016/j.foodchem.2006.09.028

Uner, N., Oruç, E. Ö., Sevgiler, Y., Şahin, N., Durmaz, H., & Usta, D. (2006). Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. *Environmental Toxicology and Pharmacology*, *21*(3), 241-245. https://doi.org/10.1016/j.etap.2005.08.007

Uney, K., Terzi, E., Corum, D. D., Ozdemir, R. C., Bilen, S., & Corum, O. (2021). Pharmacokinetics and pharmacokinetic/pharmacodynamic integration of enrofloxacin following single oral administration of different doses in brown trout (*Salmo trutta*). *Animals*, *11*, 3086. https://doi.org/10.3390/ani11113086

Uribe, C., Folch, H., Enriquez, R., & Moran, G. (2011). Innate and adaptive immunity in teleost fish: A review. *Veterinarni Medicina*, *56*, 486-503. https://doi.org/10.17221/3294-VETMED

Vaseeharan, B., & Thaya, R. (2014). Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture. *Aquaculture International*, *22*, 1079-1091. https://doi.org/10.1007/s10499-013-9729-3

Vutukuru, S. S., Chintada, S., Madhavi, K. R., Rao, J. V., Anjaneyulu, Y. (2006). Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish, *Esomus danricus*. *Fish Physiology and Biochemistry*, *32*, 221-229. https://doi.org/10.1007/s10695-006-9004-x

Waagbø, R. (1994). The impact of nutritional factors on the immune system in Atlantic salmon, *Salmo salar* L.: A review. *Aquaculture Research*, *25*(2), 175-197. https://doi.org/10.1111/j.1365-2109.1994.tb00573.x

White, P. L., Linton, C. J., Perry, M. D., Johnson, E. M., & Barnes, R. A. (2006). The evolution and evaluation of a whole blood polymerase chain reaction assay for the detection of invasive aspergillosis in hematology patients in a routine clinical setting. *Clinical Infectious Diseases*, *42*(4), 479-486. https://doi.org/10.1086/499949

Worthington, C. (1991). *Worthington Enzyme Manual*. Biochemical Freehold.

Yilmaz, O., Ozsahin, A. D., Bircan, B., Erden, Y., Karaboga, Z. (2010). Radical scavenging activity of the *Pistacia terebinthus* in fenton reagent environment and its protective effects on the unsaturated fatty acids. *Asian Journal of Chemistry*, *22*, 7949-7958.

Yılmaz, S., Ergün, S., Yiğit, M., & Yılmaz, E. (2022). An extensive review on the use of feed additives against fish diseases and improvement of health status of fish in Turkish aquaculture sector. *Aquaculture Studies*, *22*(3), AQUAST710. https://doi.org/10.4194/AQUAST710

Zahran, E., Risha, E., Abdelhamid, F., Mahgoub, H. A., Ibrahim, T. (2014). Effects of dietary *Astragalus polysaccharides* (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish & Immunology*, *38*(1), 149-157. https://doi.org/10.1016/j.fsi.2014.03.002

Zoral, M. A., Ishikawa, Y., Ohshima, T., Futami, K., Endo, M., Maita, M., & Katagiri, T. (2018). Toxicological effects and pharmacokinetics of rosemary (*Rosmarinus officinalis*) extract in common carp (*Cyprinus carpio*). *Aquaculture*, *495*, 955-960. https://doi.org/10.1016/j.aquaculture.2018.06.048

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RESEARCH ARTICLE

Preliminary determination of heavy metals in sediment, water, and some macroinvertebrates in Tawi-Tawi Bay, Philippines

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ABSTRACT

Determination of heavy metals is enormously important to determine the condition of the aquatic environment in terms of chemical pollution. In this study, a preliminary determination of heavy metal concentrations in sediment, water, and some macroinvertebrates in several sampling sites along Tawi-Tawi Bay, Philippines, was undertaken to have an initial status of heavy metal pollution in the area. Results revealed that the average concentration of heavy metals followed the order of Fe > Zn > Mn > Pb > Cu > Ni > Cd for sediment, Pb > Zn > Cu > Ni > Fe > Cd > Mn for seawater, Fe > Zn > Mn > Cu > Pb > Ni > Cd for spider conch (*Lambis lambis* Linnaeus, 1758), and Fe> Zn > Pb > Ni > Mn > Cu > Cd for sea cucumber (*Holothuria scabra,* Jaeger, 1833). However, all these determined heavy metals were within the safety limits set by WHO, US (EPA and FDA), and EMA. This study suggests that despite the anthropogenic activities in the coastal areas, heavy metal contamination in Tawi-Tawi Bay has not exceeded the safety limits.

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Introduction

Potential sources for heavy metal pollution in the aquatic environment include but not limited to mining of metals, geochemical structure, and industrial wastes (Lee & Stuebing, 1990; Gümgüm et al., 1994; Sönmez et al., 2021). Moreover, heavy metals can accumulate to toxic concentrations under some environmental conditions (Güven et al., 1999) and cause ecological harm (Harms, 1975; Jefferies,1984; Freedman, 1989).

Tawi-Tawi is located at the southern tip of the Philippines, known to be at the center of the coral triangle, endowed with great marine biodiversity. Tawi-Tawi Bay serves as the major source of juvenile fish and macroinvertebrates recruits for much of the focal areas, perhaps even for areas farther east. The bay harbors different species of macroinvertebrates that include gastropods and haliotids (Campos et al., 2007).

This bay serves as the spawning ground of all aquatic organisms, including pelagic fishes such as tunas. However, this bay also serves as a sink for a series of anthropogenic activities coming from different coastal areas. Prominent activities undertaken in this bay are mariculture, urbanization, and nickel mining activities that were recently established within the mainland of Tawi-Tawi. Seaweed farming is the main livelihood of most people thriving in these areas. To increase their seaweed production, seaweed farmers immerse their seaweeds in agricultural fertilizers (Tahiluddin et al., 2021) that have a potential source of heavy metals. Mining activities in mainland Tawi-Tawi will find their way into the bay through sedimentation. Fine sediments are known to harbor heavy metals due to their size and adhesive properties (Hwang et al., 2016). In addition, heavy maritime traffic and deep-water movements in the region are also seen as important factors that can contribute to the chemical pollution.

To our knowledge, there has been no study investigating heavy metals in water, sediment, and macroinvertebrates (spider conch and sea cucumber) in Tawi Tawi Bay, Philippines, to date. To evaluate the metal pollution of the area, we aimed to determine the heavy metal contents (Cd, Cu, Fe, Mn, Ni, Zn, and Pb) in sediment, water, and macroinvertebrates originating from the coastal region of Tawi-Tawi bay. Further comparisons with different reported data were also conducted. Our results may help in evaluating the heavy metal pollution status of Tawi-Tawi bay, Philippines, regarding the quality of macroinvertebrates, water, and sediment.

Materials and Methods

Sampling Procedure

All samples were collected in four different coastal areas (Bongao, Panglima Sugala, Banaran, and Simunul) in October 2019 within Tawi-Tawi Bay, Philippines (Fig. 1). Although Tawi-Tawi is not much affected by season, October is the peak of the wet season. Samples were collected in triplicate at each sampling site. Three individuals of spider conch and sea cucumber were collected in each sampling area. Likewise, three samples of sediment and surface water were collected in areas where the macroinvertebrates were found.

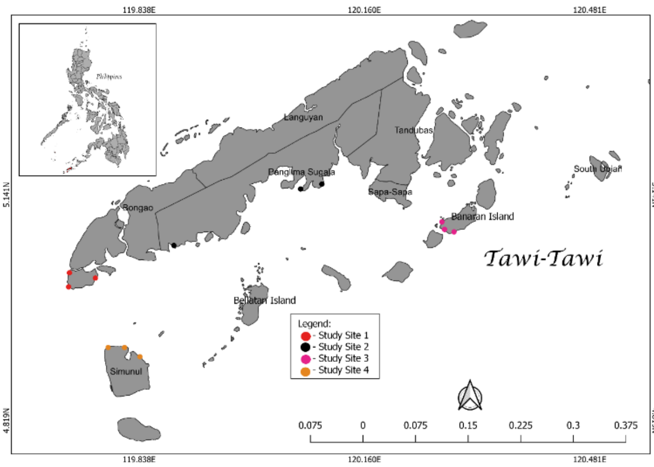

Figure 1. Map of the study sites

Water samples were collected from approximately 30 cm below the surface by immersing polyethylene bottles that were previously washed with 10% nitric acid (v/v) and rinsed thoroughly with distilled water. After collection, samples were treated with 10% nitric acid until obtaining the pH level of 2, filtered through 0.45 μm filter and stored at +4°C (Alam et al., 2001; Sönmez et al., 2012; Elderwish et al., 2019). Sediment samples were collected using a plastic shovel from the bottom (<5 cm) and placed into polyethylene ziplocked bags. The samples were then air dried in the laboratory. After drying, they were powdered, filtered through a 160-μm sieve and stored at -20°C (Öztürk et al., 2009). Macroinvertebrate samples, on the other hand, were collected by hand while freediving. Macroinvertebrate samples were washed with deionized water and stored in individual polyethylene bags at -20°C until transportation (Baharom & Ishak, 2015).

Heavy Metal Analyses

Water, sediment, and macroinvertebrate (spider conch and sea cucumber) samples were transported to Turkey by plane in a styrofoam box containing dry ice and analyzed in the Central

Research Laboratory, Kastamonu University, Turkey. Heavy metal (Cd, Cu, Fe, Mn, Ni, Zn, and Pb) concentrations in the water samples were measured directly. The heavy metal contents of the sediments were determined by digesting samples (0.5 g each) in a microwave oven for 1 h at 600 W using a high-pressure digestion vessel with 6 ml of HNO_3/HCl (v/v 2:1) as per Morillo et al. (2004). Macroinvertebrates were analyzed following the method of Sönmez et al. (2012). Briefly, samples were accurately homogenized, weighed (5 g, wet weight), and were digested with nitric acid-hydrogen peroxide (2:3) by exposing samples to 40 bar pressure microwave wet incineration units (Berghof speedwave MWS-2) in three different steps: (i) 75% microwave power for 5 min at 145°C, (ii) 90% microwave power for 10 min at 180°C, and (iii) 40% microwave power for 10 min at 100°C. The samples were then read in two parallel. All readings were conducted using inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer, Optima 2100 DV). Deionized water was used and all reagents used were of analytical grade. All glassware and plastic materials were washed using nitric acid for 15 minutes and were shaken using deionized water before use. The inert gas used was high purity argon. Standard solutions were prepared using stock solutions (Merck, multiple element standard). DORM-3 and DOLT-4 (National Research Council Canada, Ottawa, Canada) were used as certified reference materials.

Statistical Analysis

The data obtained were analyzed using a one-way analysis of variance (ANOVA). Levene's test was used for homogeneity analysis. Significant differences among sampling sites were further interpreted using Duncan's Multiple Range Test (DMRT). Data were presented in mean ± SE. All significant levels were set at 0.05.

Results

The obtained levels for the Cd, Cu, Fe, Mn, Ni, Zn, and Pb in the sediment, water, spider conch, and sea cucumber in the coastal areas of Tawi-Tawi Bay, Philippines are summarized in Tables 1, 2, 3, and 4, respectively.

All heavy metals evaluated in sediments showed a statistically significant difference ($p<0.05$) in all experimental sites (Table 1). The Bongao area had the highest concentration of all heavy metals ($p<0.05$). However, the concentration of heavy metals (Mn, Cu, Zn, Pb, and Ni) found in sediment is considered a non-polluted category (Abbasi & Mirekhtiary, 2020). In general, the accumulated levels of heavy metals in sediment were in the following order: Fe > Zn > Mn > Pb > Cu > Ni > Cd. Fe was the most abundant metal, while the lowest abundance was observed for Cd.

The seawater samples collected from different experimental sites showed a statistically significant difference in the levels of Cd, Cu, Fe, Zn, Mn, Ni concentration ($p<0.05$) but not in Pb ($p>0.05$) as shown in Table 2. The highest level of cadmium, iron, manganese, and zinc content was recorded in seawater collected from Simunul. At the same time, the highest copper level was manifested by the seawater collected from the Panglima Sugala and Simunul areas ($p<0.05$). However, the highest Ni level was recorded at Bongao station. In general, the accumulated levels of heavy metals in seawater were in the following order: Pb > Zn > Cu > Ni > Fe > Cd > Mn. Pb was the most abundant metal, while the lowest abundance was observed for Mn.

The results showed that heavy metals (Cu, Fe, Mn, Ni, Zn, and Pb) in different coastal areas of Tawi-Tawi Bay differed significantly ($p>0.05$) in spider conch except for Mn (Table 3). Spider conch *L. lambis* collected in Panglima Sugala had the highest Cd level (1.48±0.18) compared to other coastal areas

Table 1. Heavy metal concentrations (ppm, dry weight) in sediment samples collected from different coastal areas of Tawi-Tawi bay

Sites	Cd	Cu	Fe	Mn	Ni	Zn	Pb
1	$0.45±0.01^{a}$	$20.17±0.36^{a}$	$9,948±113^{a}$	$34.66±0.45^{a}$	$6.40±0.14^{a}$	$76.99±2.21^{a}$	$23.79±0.36^{a}$
2	$0.29±0.00^{c}$	$10.41±0.43^{b}$	$4,257±177^{c}$	$20.17±0.08^{b}$	$4.53±0.08^{b}$	$39.01±1.90^{c}$	$14.99±0.39^{b}$
3	$0.34±0.02^{b}$	$10.37±0.81^{b}$	$4,443±535^{c}$	$25.69±3.85^{b}$	$4.24±0.12^{b}$	$56.85±6.03^{b}$	$14.98±0.41^{b}$
4	$0.29±0.01^{c}$	$11.84±0.48^{b}$	$6,709±55^{b}$	$25.86±0.57^{b}$	$4.32±0.19^{b}$	$32.02±3.32^{c}$	$16.04±0.75^{b}$

Note: Data are presented as mean±standard error (n:9). Different superscript letters in the same column indicate significant differences between sampling sites ($p<0.05$). 1: Bongao, 2: Panglima Sugala, 3: Banaran, 4: Simunul.

Table 2. Heavy metal concentrations (ppb) in water samples collected from different coastal areas of Tawi-Tawi bay

Sites	Cd	Cu	Fe	Mn	Ni	Zn	Pb
1	0.85±0.01[b]	18.91±0.29[b]	0.54±0.09[b]	0.79±0.01[b]	11.44±0.03[a]	19.37±0.25[b]	32.92±0.12
2	0.84±0.01[b]	24.33±0.71[a]	2.00±0.26[b]	0.55±0.02[b]	11.03±0.07[b]	25.18±0.21[b]	33.92±1.07
3	0.85±0.01[b]	20.40±0.53[b]	0.95±0.07[b]	0.74±0.01[b]	11.34±0.06[ab]	21.57±0.62[b]	38.86±2.56
4	0.90±0.01[a]	22.70±0.60[a]	10.40±2.71[a]	1.31±0.23[a]	11.32±0.14[ab]	316.63±68.19[a]	35.47±2.98

Note: Data are presented as mean±standard error (n:9). Different superscript letters in the same column indicate significant differences between sampling sites (p<0.05). 1: Bongao, 2: Panglima Sugala, 3: Banaran, 4: Simunul.

Table 3. Heavy metal concentrations (ppm, wet weight) in spider conch (*Lambis lambis*) samples collected from different coastal areas of Tawi-Tawi bay

Site	Cd	Cu	Fe	Mn	Ni	Zn	Pb
1	0.87±0.09[b]	3.43±0.57[ab]	42.19±1.26[b]	9.85±0.26	1.00±0.06[b]	21.42±0.62[b]	1.21±0.02[b]
2	1.48±0.05[a]	6.06±1.13[a]	138.08±19.78[a]	9.01±0.43	1.74±0.18[a]	36.16±2.43[ab]	1.31±0.06[ab]
3	0.83±0.05[b]	2.13±0.24[b]	49.87±8.18[b]	7.87±0.48	1.02±0.01[b]	24.30±0.80[b]	1.52±0.13[a]
4	0.43±0.10[c]	4.92±1.11[ab]	65.83±20.64[b]	8.03±2.17	0.50±0.16[c]	50.62±10.39[a]	1.23±0.03[b]

Note: Data are presented as mean±standard error (n:9). Different superscript letters in the same column indicate significant differences between sampling sites (p<0.05). 1: Bongao, 2: Panglima Sugala, 3: Banaran, 4: Simunul.

Table 4. Heavy metal concentrations (ppm, wet weight) in sea cucumber (*Holothuria scabra*) samples collected from different coastal areas of Tawi-Tawi bay

Sites	Cd	Cu	Fe	Mn	Ni	Zn	Pb
1	0.06±0.01[b]	0.99±0.12[ab]	132.66±16.73[b]	0.78±0.06[c]	1.06±0.06[ab]	186.48±28.30[b]	3.58±0.02[a]
2	0.10±0.00[a]	1.38±0.23[a]	319.78±64.80[ab]	1.57±0.21[a]	0.89±0.03[b]	88.70±4.65[b]	3.58±0.15[a]
3	0.08±0.00[ab]	0.79±0.10[b]	462.02±94.12[a]	1.48±0.24[ab]	0.96±0.02[ab]	261.18±102.08[ab]	3.40±0.07[ab]
4	0.08±0.00[ab]	0.68±0.02[b]	159.54±10.61[b]	0.84±0.09[bc]	1.56±0.31[a]	573.18±141.34[a]	3.17±0.07[b]

Note: Data are presented as mean±standard error (n:9). Different superscript letters in the same column indicate significant differences between sampling sites (p<0.05). 1: Bongao, 2: Panglima Sugala, 3: Banaran, 4: Simunul.

within Tawi-Tawi Bay (p<0.05). Cu, Fe, and Ni results were also higher at station 2, similar to Cd values. The Zn value was measured as highest at the 4th station, and the Pb value was the highest at the 3rd station. In general, the accumulated levels of heavy metals in spider conch were in the following order: Fe > Zn > Mn > Cu > Pb > Ni > Cd. Fe was the most abundant metal, while the lowest abundance was observed for Cd.

Heavy metal results in sea cucumber (*H. scabra*) samples were statistically different at all stations (Table 4). Cd, Cu, Mn, and Pb levels were recorded as the highest in the second station. On the other hand, Fe was highest at the third station and Ni at the fourth station. In general, the accumulated levels of heavy metals in sea cucumber were in the following order: Fe> Zn > Pb > Ni > Mn > Cu > Cd. Fe was the most abundant metal, while the lowest abundance was observed for Cd.

Discussion

This study provides a preliminary determination of heavy metals in sediment, water, and macroinvertebrates in Tawi-Tawi Bay, Philippines. Our results revealed that the heavy metals (Cu, Fe, Mn, Ni, Zn & Pb) determined from different collected samples varied as presented in Tables 1, 2, 3, and 4. However, heavy metals in all samples were within the safety limits set by WHO, US (EPA and FDA), and EMA.

The average heavy metal content in the sediment samples decreased in the order of Fe > Zn > Mn > Pb > Cu > Ni > Cd. Fe content of Tawi-Tawi Bay's sediment ranged from 4,257-9,948 ppm, much greater than sediment determined in Quangzhou Bay (3.17 ppm, Yan et al., 2020) but considerably lower than the sediment sampled from Ryder Bay (14,900-26,300 ppm, Webb et al., 2020). The concentration of Zn in sediments of Tawi-Tawi Bay ranged from 32.02-76.99 ppm. In other studies, Zn concentrations vary according to the location; 7.6-145.9 in Monastir Bay (Amor et al., 2020), 48.6-538.2 ppm in Thessaloniki Bay (Christophoridis et al., 2019), 31.1-54.3 ppm in Ryder Bay (Webb et al., 2020), 95.2-443.4 ppm in Masan Bay (Lim et al., 2013), and 130.86 ppm in Quangzhou Bay (Yan et al., 2020). Mn content (20.17-34.66 ppm) in the sediment of Tawi-Tawi Bay is comparatively lower than other studies; 141.5-546.8 ppm in Thessaloniki Bay (Christophoridis et al., 2019), 211-404 ppm in Ryder Bay (Webb et al., 2020), and 612.6 ppm in Quangzhou Bay (Yan et al., 2020). Pb concentration (14.98-23.79 ppm) of the sediment obtained in the present study is relatively lesser than in Quangzhou Bay (42.18 ppm, Yan et al., 2020), Thessaloniki Bay (29.4-195.4 ppm, Christophoridis et al., 2019), and Masan Bay (29.0-82.5 ppm, Lim et al., 2013); however, it is higher than those obtained from Ryder Bay (4.8-5.0 ppm, Webb et al., 2020). Besides, the Pb concentration of Monastir Bay ranged from 0-47 ppm (Amor et al., 2020). Cu concentration sampled from the sediment of Tawi-Tawi Bay ranged from 10.37-20.17 ppm. In other Bays around the world, Cu concentrations in the sediments were 1.6-54.2 ppm in Monastir Bay (Amor et al., 2020), 21.3-180.1 ppm in Thessaloniki Bay (Christophoridis et al., 2019), 21.6-113.9 ppm in Masan Bay (Lim et al., 2013), 19.79 ppm in Quangzhou Bay (Yan et al., 2020), and 19.16-44.3 ppm in Ryder Bay (Webb et al., 2020). Ni concentrations in the sediment of the present study ranged from 4.24-6.4 ppm, which were relatively lower than those sampled from Thessaloniki Bay (41.8-171.3 ppm, Christophoridis et al., 2019), Masan Bay (15.5-46.6 ppm, Lim et al., 2013), and Quangzhou Bay (20.89 ppm, Yan et al., 2020). In addition, Ni concentrations in the sediments of Monastir Bay and Ryder Bay were 0.5-64.85 and 5.8-11.2 ppm, respectively (Webb et al., 2020; Amor et al., 2020). All heavy metals in the sediment were found to be higher in the first sampling site (Bongao) than any other site. Bongao is the most developed region among the sampling sites and it is inhabited by more people than others. The reason for high heavy metal concentration could be attributed to the high population and industrialization which may have led to increased anthropogenic activity.

The average heavy metal concentration in seawater samples was observed in the order of Pb > Zn > Cu > Ni > Fe > Cd > Mn. Pb concentrations determined from the coastal waters of Tawi-Tawi Bay ranged from 32.92-38.86 ppb, which are comparable to those sampled from Sheyang Estuary (0.15-1.57 ppb, Zhao et al., 2018), East GD coastal regions (0.0-7.7 ppb, Zhang et al., 2015), and West GD coastal regions (0.1-6.5 ppb, Zhang et al., 2015). Also, the Pb concentrations in the water of the Gulf of Cambay ranged from 27-2,203 ppb (Reddy et al., 2005). Cu levels (18.91-24.33 ppb) obtained from the water in the present study are relatively higher than those in Sheyang Estuary (1.94-7.39 ppb, Zhao et al., 2018), East GD coastal regions (0.2-9.3 ppb, Zhang et al., 2015), and West GD coastal regions (0.3-18.1 ppb, Zhang et al., 2015) but much lower compared to Gulf of Cambay (27-4,062 ppb, Reddy et al., 2005). Ni and Fe concentrations determined in the coastal waters of Tawi-Tawi Bay ranged from 11.03-11.44 and 0.54-10.4 ppb, relatively lower than those in the Gulf of Cambay with a concentration 23-1,076 and 24-3,785 ppb, respectively (Reddy et al., 2005). Cd concentrations in seawater samples in this study ranged from 0.84-0.9 ppb, which are greater than other studies such as 0.04-0.30 ppb in Sheyang Estuary (Zhao et al., 2018), 0.01-0.66 ppb in East GD coastal regions (Zhang et al., 2015), and 0.01-0.89 ppb in West GD coastal regions (Zhang et al., 2015), but comparably lesser than those in Gulf of Cambay with a concentration range from 27-4,062 ppb (Reddy et al., 2005). Mn concentrations in the water samples of Tawi-Tawi Bay ranged from 0.55-1.31 ppb, which are considerably lesser than those in the Gulf of Cambay (Reddy et al., 2005) with a concentration range from 25-5,152 ppb. Surprisingly, contrary to the sediment results, the heavy metals in the water except Ni and Pb were found to be higher in the fourth sampling site (Simunul) than the other sites. This could, somehow, be the result of a spontaneous pollution event that emerged in the region during the sampling period. However, it is inconclusive whether such event occurred. This emphasizes the need for a periodical study that determines the heavy metal concentrations at certain intervals in the region.

The heavy metals content in spider conch showed a trend in an order of Fe > Zn > Mn > Cu > Pb > Ni > Cd. Fe content (42.19-138.08 ppm) in spider conch was found to be considerably lesser than in limpet with a 1048-2866 ppm concentration (Webb et al., 2020). Zn content in spider conch in the present study ranged from 21.42-50.62 ppm. Gastropods normally contain Zn ranging from 2.2-60.5 ppm (Cubadda et al., 2001). Limpets, in particular, contain varying Zn contents such as 40.3-158 ppm (Pérez et al., 2019) and 61.2-82.6 ppm (Webb et al., 2020). Mn content in spider conch obtained in this study ranged from 7.87-9.85 ppm, while in limpets, it ranged from 8.8-41.9 ppm (Webb et al., 2020). Cu content in spider conch in this study ranged from 2.13-6.06 ppm. Cu content in gastropods ranges from 0.47-34.7 ppm (Cubadda et al., 2001).

Shellfish and limpets have been reported to contain Cu content ranging from 0.9-137 ppm (El Nemr et al., 2016) and 1.49-25.2 ppm, respectively (Pérez et al., 2019; Webb et al., 2020). Pb content in gastropods ranges from 0.06-2.18 ppm (Cubadda et al., 2001). In this study, Pb concentration in spider conch ranged from 1.21-1.52 ppm. Pb content in limpets was previously determined, ranging from 0.5-2.2 ppm (Webb et al., 2020) to 0.68-2.75 (Pérez et al., 2019). In shellfish, Pb concentration ranged from 0.2-17 ppm (El Nemr et al., 2016). Ni content (0.5-1.74 ppm) in spider conch was found to be relatively lower compared to other studies such as in shellfish (7.8-41 ppm, El Nemr et al., 2016) and limpets (0.82-8.4, Pérez et al., 2019; Webb et al., 2020). Cd content in spider conch determined in this study ranged from 0.43-1.48 ppm. These values are within the values of Cd content in gastropods (0.1-6.6 ppm, Cubadda et al., 2001); however, they are higher than those reported in limpets with 1.49-25.2 ppm concentrations (Pérez et al., 2019; Webb et al., 2020). In addition, Cd content in shellfish ranged from 0.04-1.7 ppm (El Nemr et al., 2016).

The mean heavy metal content in the sea cucumber samples (*H. Scabra*) followed the order of Fe> Zn > Pb > Ni > Mn > Cu > Cd. Fe content (132.66-462.02 ppm) in *H. scabra* obtained from the present study was determined to be relatively higher than those obtained from other sea cucumber species such as in *Stichopus herrmanni* with a concentration of 14.6 ppm (de Fretes et al., 2020), in *H. edulis* with a concentration of 39.82 ppm (Jinadasa et al., 2014) and in *H. atra* with a concentration of 11.72 ppm (Jinadasa et al., 2014). Zn content (88.7-573.18 ppm) in *H. scabra* was also found to be comparably greater than those other sea cucumber species; 2.634 ppm in *Apostichopus japonicas* (Jiang et al., 2015), 20.95 ppm in *H. edulis* (Jinadasa et al., 2014), 20.30-36.21 ppm in *A. japonicas* (Mohsen et al., 2019), and 24.38 ppm in *H. atra* (Jinadasa et al., 2014). Pb content (3.17-3.58 ppm) in *H. scabra* was found to be much higher than in *A. japonicas* (Jiang et al., 2015), *H. edulis* (Jinadasa et al., 2014), and *H. atra* (Jinadasa et al., 2014) with concentrations of 0.065, 0.03, 0.1 ppm, respectively. Ni content (0.89-1.56) in *H. scabra* was determined to be relatively similar to *A. japonicas* with a concentration of 1.18-1.77 ppm (Mohsen et al., 2019). Mn content (0.78-1.57 ppm) in *H. scabra* was observed to be comparably lower than *A. japonicas* with 16.37-58.91 ppm concentrations (Mohsen et al., 2019). Cu content (0.68-1.38 ppm) in *H. scabra* was found to be much lower compared to *H. atra* (3.18 ppm, Jinadasa et al., 2014), *A. japonicas* (1.55-8.21 ppm, Mohsen et al., 2019), *H. edulis* (1.84 ppm, Jinadasa et al., 2014), however, it was determined to be higher than in *A. japonicas* (0.179 ppm, Jiang et al., 2015). Cd content (0.06-0.1 ppm) in *H. scabra* was observed to be much lesser than in *A. japonicas* (0.161-0.85 ppm, Jiang et al., 2015; Mohsen et al., 2019), and relatively similar with *H. edulis* (0.11 pm, Jinadasa et al., 2014) and *H. atra* (0.07 ppm, Jinadasa et al., 2014). In general, these results suggest that contents of heavy metals in sea cucumber from the coastal area of Tawi-Tawi are similar, lower, or higher than previously reported contents. It should be noted that the sea cucumbers inhabiting different areas were compared and the heavy metal contents among sampling sites varied. However, the chemical contents between different species as well as individuals of the same species are under the influence of many factors including but not limited to collection site and time, gender, habitat, age, feeding habits, etc. (Morgano et al., 2011). Nonetheless, to our knowledge, there has been no study regarding the heavy metal contents in Tawitawi Bay, Philippines, and the reliable data concerning the heavy metals in sea cucumbers and spider conch are also limited.

Several reports designating anthropogenic activities as the main source of heavy metals contamination have been documented in different parts of the globe. Like in the case of metal concentrations in Korean coastal sediment, which are significantly influenced by human activities associated with urbanization (Hwang et al., 2016). The same report on heavy metals in the nearshore sediment of Daya Bay was all closely related to the import of anthropogenic activities (Qu et al., 2018). Anthropogenic influences on heavy metals accumulation were also observed along the Karachi coast, Pakistan (Saher & Siddique, 2019). Likewise, anthropogenic sources of contamination of heavy metals such as Zn, Cd, Cu, and Pb were also evident in the coastal sediments from the coastal areas of the Bohai Sea and the Yellow Sea (Tian et al., 2020). Sediments of Edku at the Deltaic coast of Egypt receiving sewage and untreated wastewater were also heavily polluted with heavy metals (Keshta et al., 2020). Influence by anthropogenic activities was also suggested as contamination and risk of heavy metals found in the northwest coastal area of the Bohai Sea (Wang et al., 2020). Domestic effluents and urban runoff were suggested as the sources of Zn contamination in New York Bight. Corrosion within the urban water supply network may contribute to the Zn levels in domestic effluents significantly. Contamination of the urban stormwater as well as zinc release from the roofs as a result of the corrosion could also elevate the Zn levels in near water sources where the runoff waters are discharged into (Amiard & Amiard-Triquet, 1993).

The correlation of anthropogenic activities with heavy metals (Cu, Ni, Pb, and Zn) was evident in marine surface sediments of the Thessaloniki Bay, Northern Greece (Christophoridis et al., 2019). Enrichment of heavy metals such as Cd, Cu, Ni, and Zn in coastal sediments of the Dammam, Al-Jubail area, Saudi Arabian Gulf was reported to be originating from anthropogenic sources (El-Sorogy et al., 2018). Wastewater discharges were also seen as one of the major contributors to heavy metal pollution in the surface sediments of Monastir Bay (Eastern Tunisia, Mediterranean Sea) (Amor et al., 2020). Sediment contamination associated with Cu, Zn, Pb, Mn, and Cd has occurred in Quanzhou Bay, southeast China since the 19th century caused by domestic emission (Yan et al., 2020). Jiangsu coastal environment in China was also affected by heavy metals pollution of Cu, Pb, Cd, and Zn, and these were strongly influenced by anthropogenic activities (Chen et al., 2020). The significant anthropogenic sources of heavy metal contamination were reported in Sabratha coastal sediments. High levels of presence in the sediment of heavy metals were defined as an anthropogenic component, as Karthikeyan et al. (2020) observed in the surface sediment of Emerald Lake, India. They found that almost all the sites were polluted by heavy metals. The anthropogenic origin of Pb contamination was also reported in the coastal sediments of the Egyptian Mediterranean coast (El Baz & Khalil, 2018). Various anthropogenic activities along the Daya Bay, South China Sea, were reported to contribute mainly to the heavy metal contamination (Liu et al., 2018).

Conclusion

In conclusion, this study revealed that the heavy metals (Cu, Fe, Mn, Ni, Zn & Pb) in the sediment, water, and macroinvertebrate samples collected from different sites along Tawi-Tawi Bay, Philippines, varied greatly. The mean concentration of heavy metals decreased in the order of Zn > Mn > Pb > Cu > Fe > Ni > Cd for sediment, Pb > Zn > Cu > Ni > Fe > Cd > Mn for seawater, Fe > Zn > Mn > Cu > Pb > Ni > Cd for spider conch, and Fe> Zn > Pb > Ni > Mn > Cu > Cd for sea cucumber. However, heavy metals in all samples in the present study were determined to be within the safety limits set by WHO, US (EPA and FDA), and EMA. This implies that despite urbanization and industrialization in the coastal areas of Tawi-Tawi province, Philippines, heavy metal levels, especially in the commercially important macroinvertebrates such as spider conch and sea cucumber, do not exceed the safety limits; hence, they cannot be considered as polluted. Moreover, it is also important to have frequent and periodical heavy metal monitoring as the other sites constantly receive heavy metal pollution pressures.

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Compliance With Ethical Standards

Authors' Contributions

AHI: Sampling, transportation of the samples, article writing
YT: Statistical analyses, article reviewing & editing
AT: Article reviewing & editing
SB: Article reviewing & editing
YUJ: Article writing
AYS: Study design, data evaluation, article reviewing & editing

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Abbasi, A., & Mirekhtiary, F. (2020). Heavy metals and natural radioactivity concentration in sediments of the Mediterranean Sea coast. *Marine Pollution Bulletin*, *154*, 111041. https://doi.org/10.1016/j.marpolbul.2020.111041

Alam, M. G. M., Tanaka, A., Stagnitti, F., Allinson, G., & Maekawa, T. (2001). Observations on the effects of caged carp culture on water and sediment metal concentrations in lake Kasumigaura, Japan. *Ecotoxicology and Environmental Safety*, *48*(1), 107-115. https://doi.org/10.1006/eesa.2000.1989

Amiard, J. C., & Amiard-Triquet, C. (1993). *Handbook of hazardous materials* (1st ed.). Academic Press.

Amor, R., Jerbi, H., Abidi, M., & Gueddari, M. (2020). Assessment of trace metal contamination, total organic carbon and nutrient accumulation in surface sediments of Monastir Bay (Eastern Tunisia, Mediterranean Sea). *Regional Studies in Marine Science*, *34*, 101089. https://doi.org/10.1016/j.rsma.2020.101089

Baharom, Z. S., & Ishak, M. Y. (2015). Determination of heavy metal accumulation in fish species in Galas River, Kelantan and Beranang mining pool, Selangor. *Procedia Environmental Sciences*, *30*, 320-325. https://doi.org/10.1016/j.proenv.2015.10.057

Campos, W. L., Villarta, K. A., Asis, J. C., Beldia II, P. D., Estremadura, D. G., Genito, G. E., Mequila, A. T., Nabuab, F. B., Noblezada, M. P., Santillan, A. S., & Tajolosa, M. T. (2007). Monitoring and Baseline Assessment of Marine Protected Areas in the Fish Project Focal Areas: Tawi-Tawi. Implementation of the Fisheries Improved for Sustainable Harvest Project, Project No.: 492-C-00-03-00022-00. University of the Philippines in the Visayas Foundation Inc.

Chen, Y., Liu, Q., Xu, M., & Wang, Z. (2020). Inter-annual variability of heavy metals pollution in surface sediment of Jiangsu coastal region, China: Case study of the Defeng port. *Marine Pollution Bulletin*, *150*, 110720. https://doi.org/10.1016/j.marpolbul.2019.110720

Christophoridis, C., Bourliva, A., Evgenakis, E., Papadopoulou, L., & Fytianos, K. (2019). Effects of anthropogenic activities on the levels of heavy metals in marine surface sediment of the Thessaloniki Bay, Northern Greece: Spatial distribution, sources and contamination assessment. *Microchemical Journal*, *149*, 104001. https://doi.org/10.1016/j.microc.2019.104001

Cubadda, F., Conti, M. E., & Campanella, L. (2001). Size-dependent concentration of trace metals in four Mediterranean gastropods. *Chemosphere*, *45*(4-5), 561-569. https://doi.org/10.1016/s0045-6535(01)00013-3

de Fretes, C. C., Kakisina, P., & Rumahlatu, D. (2020). Concentration of heavy metal Hg, Au, and Fe in sediments, water, and tissue damage of golden sea cucumber *Stichopus herrmanni* (Semper, 1868) (Holothuroidea; Stichopodidae) in Kayeli Bay, Indonesia. *Acta Aquatica Turcica*, *16*(1), 113-123. https://doi.org/10.22392/actaquatr.603602

El Baz, S. M., & Khalil, M. M. (2018). Assessment of trace metals contamination in the coastal sediments of the Egyptian Mediterranean coast. *Journal of African Earth Sciences*, *143*, 195-200. https://doi.org/10.1016/j.jafrearsci.2018.03.029

El Nemr, A., El-Said, G. F., Ragab, S., Khaled, A., & El-Sikaily, A. (2016). The distribution, contamination and risk assessment of heavy metals in sediment and shellfish from the Red Sea coast, Egypt. *Chemosphere*, *165*, 369-380. https://doi.org/10.1016/j.chemosphere.2016.09.048

Elderwish, N. M., Taştan, Y., & Sönmez, A. Y. (2019). *Türkiye'nin batı Karadeniz kıyı sularındaki ağır metal birikiminin mevsimsel olarak incelenmesi* [Seasonal investigation of heavy metal accumulation in waters of western Black Sea coasts of Turkey]. *Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi*, *5*(2), 1-8.

El-Sorogy, A., Al-Kahtany, A., Youssef, M., Al-Kahtany, F., & Al-Malky, M. (2018). Distribution and metal contamination in the coastal sediments of Dammam Al-Jubail area, Arabian Gulf, Saudi Arabia. *Marine Pollution Bulletin*, *128*, 8-16. https://doi.org/10.1016/j.marpolbul.2017.12.066

Freedman, B. (1989). *Environmental ecology: The impacts of pollution and other stresses on ecosystem structure and function*. Academic Press, Inc.

Gümgüm, B., Ünlü, E., Tez, Z., & Gülsün, Z. (1994). Heavy metal pollution in water, sediment and fish from the Tigris River in Turkey. *Chemosphere*, *29*(1), 111–116. https://doi.org/10.1016/0045-6535(94)90094-9

Güven, K., Özbay, C., Ünlü, E., & Satar, A. (1999). Acute lethal toxicity and accumulation of copper in *Gammarus pulex* (L.) (Amphipoda). *Turkish Journal of Biology*, *23*, 513–521.

Harms, U. (1975). The levels of heavy metals (Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, Hg) in fish from onshore and offshore waters of the German Bight. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *157*(3), 125-132. https://doi.org/10.1007/BF01140285

Hwang, D. W., Kim, S. G., Choi, M., Lee, I. S., Kim, S. S., & Choi, H. G. (2016). Monitoring of trace metals in coastal sediments around Korean Peninsula. *Marine Pollution Bulletin*, *102*(1), 230-239. https://doi.org/10.1016/j.marpolbul.2015.09.045

Jefferies, D. J. (1984). *Chemical analysis of some coarse fish from a Suffolk River carried out as part of the preparation for the first release of captive-bred otters.*

Jiang, H., Tang, S., Qin, D., Chen, Z., Wang, J., Bai, S., & Mou, Z. (2015). Heavy metals in sea cucumber juveniles from coastal areas of Bohai and Yellow Seas, North China. *Bulletin of Environmental Contamination and Toxicology*, *94*(5), 577-582. https://doi.org/10.1007/s00128-014-1432-1

Jinadasa, B. K. K. K., Samanthi, R. I., & Wicramsinghe, I. (2014). Trace metal accumulation in tissue of sea cucumber species; North-Western Sea of Sri Lanka. *American Journal of Public Health Research*, *2*(5A), 1-5.

Karthikeyan, P., Vennila, G., Nanthakumar, G., & Aswini, M. (2020). Dataset for spatial distribution and pollution indices of heavy metals in the surface sediments of Emerald Lake, Tamil Nadu, India. *Data in Brief*, *28*, 104877. https://doi.org/10.1016/j.dib.2019.104877

Keshta, A. E., Shaltout, K. H., Baldwin, A. H., Sharaf El-Din, A. A. (2020). Sediment clays are trapping heavy metals in urban lakes: An indicator for severe industrial and agricultural influence on coastal wetlands at the Mediterranean coast of Egypt. *Marine Pollution Bulletin*, *151*, 110816. https://doi.org/10.1016/j.marpolbul.2019.110816

Lee, Y. H., & Stuebing, R. B. (1990). Heavy metal contamination in the River Toad, *Bufo juxtasper* (Inger), near a copper mine in East Malaysia. *Bulletin of Environmental Contamination and Toxicology*, *45*(2), 272–279. https://doi.org/10.1007/BF01700195

Lim, D., Choi, J., Shin, H. H., Jeong, D. H., & Jung, H. S. (2013). Toxicological impact assessment of heavy metal contamination on macrobenthic communities in southern coastal sediments of Korea. *Marine Pollution Bulletin*, *73*(1), 362-368. https://doi.org/10.1016/j.marpolbul.2013.05.037

Liu, J. J., Ni, Z. X., Diao, Z. H., Hu, Y. X., & Xu, X. R. (2018). Contamination level, chemical fraction and ecological risk of heavy metals in sediments from Daya Bay, South China Sea. *Marine Pollution Bulletin*, *128*, 132-139. https://doi.org/10.1016/j.marpolbul.2018.01.021

Mohsen, M., Wang, Q., Zhang, L., Sun, L., Lin, C., & Yang, H. (2019). Heavy metals in sediment, microplastic and sea cucumber *Apostichopus japonicus* from farms in China. *Marine Pollution Bulletin*, *143*, 42-49. https://doi.org/10.1016/j.marpolbul.2019.04.025

Morgano, M. A., Rabonato, L. C., Milani, R. F., Miyagusku, L., & Balian, S. C. (2011). Assessment of trace elements in fishes of Japanese foods marketed in São Paulo (Brazil). *Food Control*, *22*(5), 778-785. https://doi.org/10.1016/j.foodcont.2010.11.016

Morillo, J., Usero, J., & Gracia, I. (2004). Heavy metal distribution in marine sediments from the southwest coast of Spain. *Chemosphere*, *55*(3), 431-442. https://doi.org/10.1016/j.chemosphere.2003.10.047

Öztürk, M., Özözen, G., Minareci, O., & Minareci, E. (2009). Determination of heavy metals in fish, water and sediments of Avsar Dam Lake in Turkey. *Journal of Environmental Health Science & Engineering*, *6*(2), 73-80.

Pérez, S., Sánchez-Marín, P., Bellas, J., Viñas, L., Besada, V., & Fernández, N. (2019). Limpets (*Patella* spp. Mollusca, Gastropoda) as model organisms for biomonitoring environmental quality. *Ecological Indicators*, *101*, 150-162. https://doi.org/10.1016/j.ecolind.2019.01.016

Qu, B., Song, J., Yuan, H., Li, X., Li, N., & Duan, L. (2018). Intensive anthropogenic activities had affected Daya Bay in South China Sea since the 1980s: Evidence from heavy metal contamination. *Marine Pollution Bulletin*, *135*, 318-331. https://doi.org/10.1016/j.marpolbul.2018.07.011

Reddy, M. S., Basha, S., Joshi, H. V., & Ramachandraiah, G. (2005). Seasonal distribution and contamination levels of total PHCs, PAHs and heavy metals in coastal waters of the Alang-Sosiya ship scrapping yard, Gulf of Cambay, India. *Chemosphere*, *61*(11), 1587-1593. https://doi.org/10.1016/j.chemosphere.2005.04.093

Saher, N. U., & Siddique, A. S. (2019). Occurrence of heavy metals in sediments and their bioaccumulation in sentinel crab *(Macrophthalmus depressus)* from highly impacted coastal zone. *Chemosphere*, *221*, 89-98. https://doi.org/10.1016/j.chemosphere.2019.01.008

Sönmez, A. Y., Karataş, M., Arslan, G., & Aras, M. S. (2021). *Sular bilgisi* (2nd ed.). Nobel Yayınevi.

Sönmez, A. Y., Yağanoğlu, A. M., Arslan, G., & Hisar, O. (2012). Metals in two species of fish in Karasu river. *Bulletin of Environmental Contamination and Toxicology*, *89*(6), 1190-1195. https://doi.org/10.1007/s00128-012-0841-2

Tahiluddin, A. B., Nuñal, S. N., Luhan, M. R. J., & Santander–de Leon, S. M. S. (2021). *Vibrio* and heterotrophic marine bacteria composition and abundance in nutrient-enriched *Kappaphycus striatus*. *Philippine Journal of Science*, *150*(6B), 1751-1763.

Tian, K., Wu, Q., Liu, P., Hu, W., Huang, B., Shi, B., Zhou, Y., Kwon, B., Choi, K., Ryu, J., & Khim, J. (2020). Ecological risk assessment of heavy metals in sediments and water from the coastal areas of the Bohai Sea and the Yellow Sea. *Environment International*, *136*, 105512. https://doi.org/10.1016/j.envint.2020.105512

Wang, X., Fu, R., Li, H., Zhang, Y., Lu, M., Xiao, K., Zhang, X., Zheng, C., & Xiong, Y. (2020). Heavy metal contamination in surface sediments: A comprehensive, large-scale evaluation for the Bohai Sea, China. *Environmental Pollution*, *260*, 113986. https://doi.org/10.1016/j.envpol.2020.113986

Webb, A. L., Hughes, K. A., Grand, M. M., Lohan, M. C., & Peck, L. S. (2020). Sources of elevated heavy metal concentrations in sediment and benthic marine invertebrates of the western Antarctic Peninsula. *Science of The Total Environment*, *698*, 134268. https://doi.org/10.1016/j.scitotenv.2019.134268

Yan, Y., Han, L., Yu, R., Hu, G., Zhang, W., Cui, J., Yan, Y., & Hung, H. (2020). Background determination, pollution assessment and source analysis of heavy metals in estuarine sediments from Quanzhou Bay, southeast China. *Catena*, *187*, 104322. https://doi.org/10.1016/j.catena.2019.104322

Zhang, L., Shi, Z., Zhang, J., Jiang, Z., Wang, F., & Huang, X. (2015). Spatial and seasonal characteristics of dissolved heavy metals in the east and west Guangdong coastal waters, South China. *Marine Pollution Bulletin*, *95*(1), 419-426. https://doi.org/10.1016/j.marpolbul.2015.03.035

Zhao, Y., Xu, M., Liu, Q., Wang, Z., Zhao, L., & Chen, Y. (2018). Study of heavy metal pollution, ecological risk and source apportionment in the surface water and sediment of the Jiangsu coastal region, China: A case study of the Sheyang Estuary. *Marine Pollution Bulletin*, *137*, 601-609. https://doi.org/10.1016/j.marpolbul.2018.10.044

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RESEARCH ARTICLE

A bibliometric review and science mapping research of oil spill response

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ABSTRACT

This study aimed to evaluate the research output of oil spill response and citations from 2000 to 2022 through a bibliometric analysis. The primary findings were as follows: there has been a noticeable increase in the number of publications. The most active journal was Marine Pollution Bulletin. Environmental sciences ranked as the most popular subject area. The United States of America (USA) published the greatest number of single-authored, internationally collaborative, first-authored papers. Oil spill(s) was the most widely queried research term, ranking first in the article title, abstract, and author keyword analysis, respectively. The Deep-Water Horizon disaster, the largest marine oil spill in 2010, was the most frequently analyzed oil spill accident in the research as a sample. This study makes a significant contribution to the field of oil spill response science by being one of the few that applies network visualization and mapping technique. Further research is recommended in light of longer-term data and the diverse Web of Science (WoS) categories found in oil spill science, which may be visualized using a variety of bibliometric visualization applications.

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Introduction

Oil, as a significant factor affecting economic development and human life (Prendergast & Gschwend, 2014) has increased the worldwide seaborne trade of oil via tankers, as well as the oil exploration and production industries, resulting in significant benefits for diverse locales, regions, and nations (Hung et al., 2018; Wang et al., 2022). According to data findings (Sirimanne et al., 2019), oil tanker deadweight tonnage increased from 0.337 billion in 1980 to 0.601 billion in 2020, while the number of oil tankers increased from 10,609 in 2010 to 11,268 in 2020. However, as oil transportation increased, the risk of oil spills affecting marine ecosystems and coastal resources enhanced, resulting in the loss of fisheries,

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aquaculture, and tourism industries, as well as severe damage to social and environmental values (Li et al., 2016; Hung et al., 2018; Chen et al., 2019). According to ITOPF (2021) statistical data report, statistics on tanker spills more than 7 tones have showed a substantial decline over the last half-century. However, tanker collisions resulted in roughly 10,000 tons of oil being lost to the environment in 2021. This is a significant increase over the prior two years, owing mostly to the one large occurrence oil spill accident reported. Nonetheless, despite significant technological advancements aimed at ensuring the safety of navigation in order to minimize the likelihood of oil leakage (Li et al., 2016; Bucelli et al., 2018), the possibility and severity of oil spills at sea cannot be completely ruled out, as evidenced by several recent severe accidents (Bonvicini et al., 2022; Wang et al., 2022). To mitigate these consequences, preparedness and response to any oil spill are always necessary and important for sensitive resources, including observation, detection, mitigation, response, and remediation of oil pollution (Li et al., 2016; Hung et al., 2018).

Despite the devastating effects of oil spills on marine ecosystems, social and economic life, and worldwide policies encouraging scientific research on the subject, the peer-reviewed literature on oil spill response systems is expected to widen in the future years (Neves et al., 2015; Murphy et al., 2016). Although many studies and equipment exist that support oil spill response planning, because of this issue there remains a need for a comprehensive and review of oil spill research and their consequences. Current and future researchers, as well as their funding agencies, will benefit from this information, which will help them better understand the nature of oil spill response research, as well as latest trends and gaps in the field.

The application of bibliometric network analysis has proven to be an effective method for statistically assessing academic literature trends and patterns (Pauna et al., 2019). The study aims to conduct a bibliometric network analysis of the scientific literature on the subject of oil spill response research. The purposes of this study are (1) to present a detailed review of the evolution of research in these particular areas; (2) to ascertain the distribution of contributing countries and the most prolific journals on specified topics; (3) to establish the most commonly appearing keywords on the subject; and (4) to establish the most productive authors who have made major contributions.

The contribution of this research is in determining the publishing and citation trends in this subject throughout time, as well as the productive authors who have extensive research. This analysis also identifies the most productive journals that publish the results of research on oil spill response science, as well as their co-citation links. This is followed by a discussion of the characteristics of the most frequently referenced articles, which include critical information for field scholars. Additionally, the most productive countries, organizations, and authors are explored, as well as scientific cooperation at the country/organization/author level. Additionally, the co-occurrence of keyword analysis identifies the most commonly used keywords over time.

Literature Review

The shipping sector has undergone a series of large-scale ocean oil spills such as M/T Sanchi oil tanker collision (2018- estimated 138,000 tones spill size) or M/V Wakashio capesize bulk carrier grounded (2020- estimated 1,000 tones spill size). These large-scale spills in highly dynamic aquatic ecosystems emphasize the importance of rapid response technology capable of mitigating potential damage (Prendergast & Gschwend, 2014; Chen et al., 2020). To mitigate the negative consequences of an oil spill, it is also vital to manage spilled oil in an orderly and timely manner (Mohammadiun et al., 2021; Yang et al., 2021). This requires the development of both short- and long-term contingency strategies (Chang et al., 2014; Wang et al., 2022). A range of response methods are included in the oil spill control strategy/contingency, with the purpose of limiting possible damage to human health and the environment by maintaining a timely and coordinated response (Li et al., 2016). Effective monitoring techniques can help with spill cleanup by detecting slicks early and specifying oil characteristics, estimating spill volume, and predicting oil movement (Robbe & Hengstermann, 2006).

To lessen the severity of marine accidents, emergency response is critical, and considerable work has been done in this area. Krohling & Campanharo (2011) proposed a fuzzy Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) method for the selection of the best response technique to spill accident. Passos et al. (2014) presented a multi-criteria decision method for assessing oil spill risk assessment on the Brazilian shore. Su et al. (2014) studied the factors affecting emergency response ability throughout the full process of unexpected water contamination events in a systematic and complete manner, and developed an emergency capability evaluation index system model. According to Alves et al. (2015), who advocate employing chemical dispersants for emergency reaction to major unintentional oil spills in a matter of hours, The oil slick paths are impacted by prevailing winds and current factors. Li et al. (2016) aimed to provide a comprehensive review of the current situations and impacts of offshore oil spills, as well as the policies and technologies in offshore oil spill response and countermeasures. This study focused on the emerging issues in cold and harsh environments due to the increased risk of oil spills in northern regions as the Arctic Passage expands. Bonvicini et al. (2022) is performed Response Gap Analysis (RGA) methodology, which is identified the environmental factors negatively influencing the emergency response in a given sea area. Mechanical, chemical, and thermal response techniques are analyzed based on 11 environmental factors of RGA methodology. Diverse research on the oil spill science have been presented in various countries, collaborations, or contexts, thus a study focusing on a better understanding of the knowledge structure in this field is necessary. As a result, the study intends to enhance the contributions of existing publications by providing a bibliometric analysis of this research subject. This is a quantitative tool for examining the impact of a given scientific topic.

Bibliometric network visualization has been utilized in recent years to assess the research trends of many scientific categories and applications in numerous scientific domains (Zou et al., 2018; Khalid et al., 2021). These studies have shown that analyzing published literature can reveal important details about research production and scientific value. Furthermore, it is a critical issue that describing patterns and characteristics of publications for a certain scientific topic might lead to cooperation between researchers and organizations.

Despite substantial research on bibliometric analysis from a variety of fields, there are just a few studies that specifically focus on the application of bibliometric studies to oil spill science and response techniques. Mohammadiun et al. (2021) used bibliometric mapping analysis to profile computational strategies for marine oil spill control, which is a multi-stage and timely sensitive process that should be adequately prepared before any spill incidents occur, using data from the Google Scholar database. Herraro-Franco et al. (2021), presents to utilize bibliometric methods to study the network structure of studies examining the oil and environment interaction of South American countries. This research allowed for the gathering of knowledge on environmental protection, solutions, and energy alternatives, with environmental sciences accounting for 47 percent of the publications. According to Lim et al. (2021), perform a systematic review of diesel pollution studies in Antarctica published in the Scopus database, compares historical and current approaches to the management of fuel oil pollution in Antarctica and highlights the potential of plant breeding as a new hope for bioremediation response technique. Another research of the diesel contaminated seawater bioremediation response technique with Bacteria provides an up-to-date summary of the role of bioremediation in diesel hydrocarbon degradation and the impacts of oil spills on the environment and living species (Khalid et al., 2021). Vasconcelos et al. (2020) is aimed to perform network analysis of 50 year's oil spill detection and mapping publications. This study is revealed that the detection of oil in the water has evolved significantly in recent decades, and there is a close link between technological advancements aimed at detection and the advancement of remote sensing data collecting methods.

Materials and Methods

Bibliometric network analysis is a powerful method for investigating certain fields of study by combining bibliometrics and network mapping analysis (Sweileh et al., 2017; Zou et al., 2018). In bibliometric science, network mapping and cluster analysis based on network data allow the application of systematic thinking and the conduct of detailed literature reviews (Pauna et al., 2019). This type of study enables the system of a network based on the relationships between countries, publications, organizations, authors, and keywords associated with the research topic (Chen et al., 2016). The availability of numerous scholarly databases, in particular as Web of Science (WoS), Scopus, and Google Scholar, significantly facilitates the process of searching for and retrieving scientific papers for bibliometric analysis (Wong et al., 2020). WoS is widely recognized as the most authoritative database for these purposes, as it includes the highest number of indexed journals and conference papers, spanning over 150 research areas (Gonçalves et al., 2019). As a result, the WoS database was utilized as the data source for this investigation.

On February 02nd, 2022, the publications for this study were retrieved from the WoS search engine using the search terms "oil spill* AND (logic gate) response*" and any other related terms. The asterisk in the search string functioned as a wildcard operator, allowing for the addition of terms before by the asterisk. All data was stored as "Tab-delimited (Mac)" files that contained the contents of "Full Record and Cited References." The contents of "Full Record" and "Cited References" were used to conduct co-authorship and co-occurrence studies (e.g., network maps of authors, nations, and keywords), as well as citation analysis (e.g., network map of scientific journals). The search found 4620 items, which included a variety of different types of English language research papers published between 2000 and 2022 year.

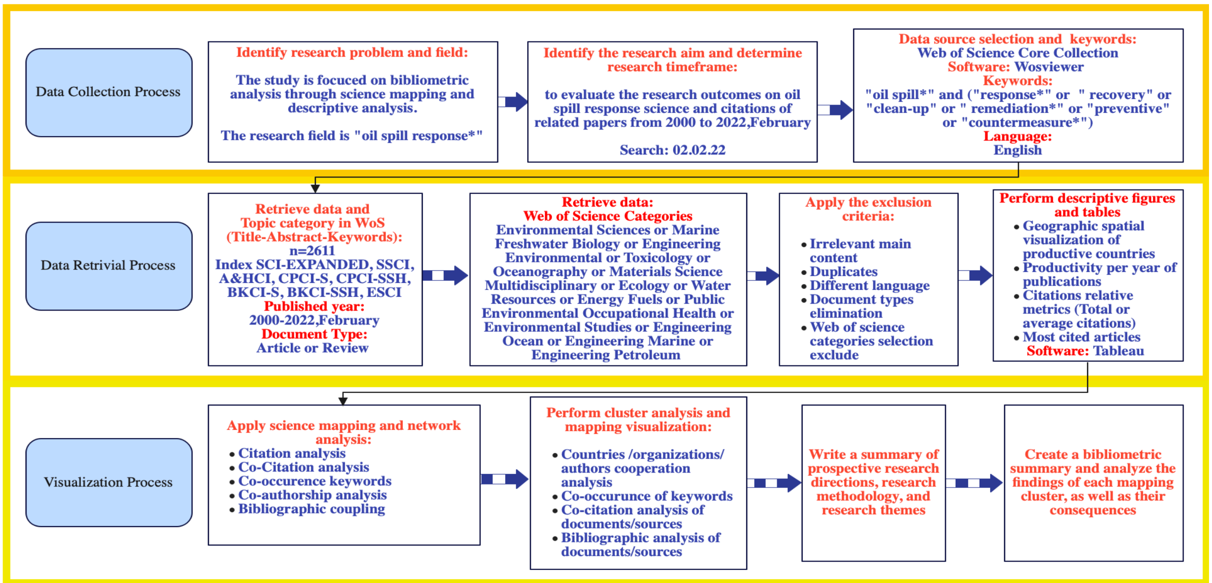

Figure 1. The conceptual framework of methodology

However, this analysis evaluated only articles and reviews with a limited WoS category, totaling 2611 publications. The WoS categories included in the research were limited to the maritime, environment and oceanographic sectors.

VOSviewer (version 1.6.18) software was utilized to conduct the bibliometric analysis in this research. This software enables users to create, visualize, and explore maps based on bibliometric network data (Van Eck & Waltman, 2013). The output findings are presented in sets to facilitate visualizing the existing relationships between bibliometric data.

The data analysis process was divided into two stages: To begin, we used Tableau software to conduct a geographic analysis of bibliometric data in order to identify the top countries by article count. The second stage was the production of bibliometric maps using the VOSviewer software, based on an examination of author co-citations, bibliographic coupling, and co-occurrence of the authors' keywords and most cited journals. A framework of the methodology, consisting of data collection, data processing and visualization stages, is shown in Figure 1.

For the aim of this study, the following terms of search results were included in the WoS queries (n=2611):

- ("oil spill*" and ("response*" or "recovery" or "clean-up" or "remediation*" or "preventive" or "countermeasure*") (Topic) and
- 2000-2022, February (Year Published) and
- Article or Review (Document Type) and
- English (Language) and
- Environmental Sciences or Marine Freshwater Biology or Engineering Environmental or Toxicology or Oceanography or Materials Science Multidisciplinary or Ecology or Water Resources or Energy Fuels or Public Environmental Occupational Health or Environmental Studies or Engineering Ocean or Engineering Marine or Engineering Petroleum (Web of Science Categories).

Finding and Results

From 2000 to 2022, all research and review articles on oil spill response were examined as follows. The aspects covered were the growth trajectory of publications and citations, the top cited of publication outputs, the distribution of publication outputs across subject categories and journals, and the publication outputs of individual countries; and the words in article titles, abstracts, and author keywords, as well as co-citation analysis, which is refers to the frequency with which two documents are mentioned by other documents. Figure 2 shows the scientific production of articles with citation analysis between 2000-2022, with a total of 2611 articles.

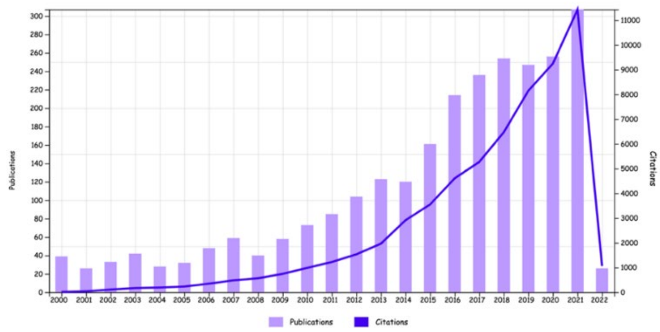

Figure 2. Times cited and publications over time

With 59 articles and 485 citations, the first peak in productivity was seen in the mid-2007s, accounting for 2.3 percent of overall productivity for the study period. Between 2016 and 2021, the highest peak of productivity was seen, with

1514 and 45260 citations respectively. This decadal period has by far the most publications, accounting for 57.9% of the total number of articles published over the length of this 20-year span. Figure 3 shows a bibliometric network to visualize collaboration nets and the distribution of publications across the world regarding the application of oil spill response query.

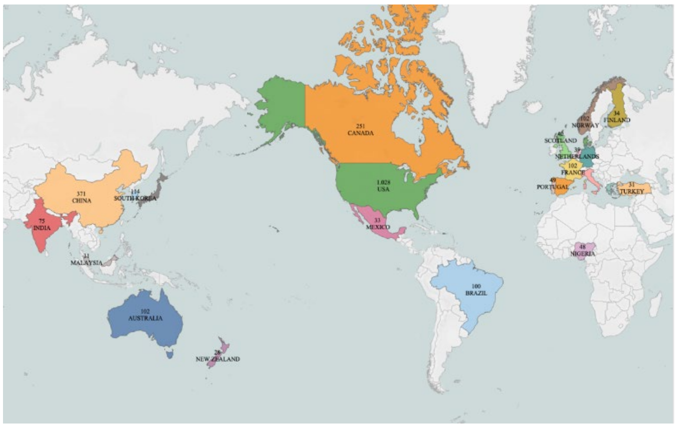

Figure 3. Most productive countries/territories of articles on oil spill response

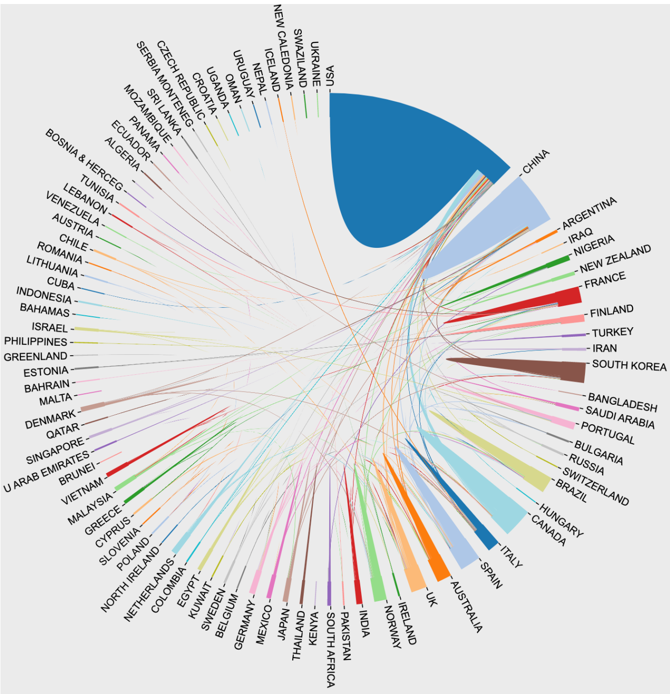

Figure 4. Interactions among the countries featured in the articles (Online platform: http://bibliometric.com/)

Results show a total of minimum 5 publications number of articles from different countries with at least one connection with each other. United States of America (USA) ranked first, with a total of 1028 (i.e., 39.3% of the total) documents, followed by China (371 docs~14.2% of the total) and third one is Canada (251 docs~9.6% of the total). Regarding the number of citations by countries, the United States ranked first, with a total of 26898(32% of the total) documents, followed respectively by China with 10017(11.9% of the total) and Spain 4341 (5.17% of the total). Figure 4 shows interactions among the countries related to oil spill response articles.

As shown in Figure 4, the most intensive link strength was between the USA and China, followed by the USA and Canada. These linkages are stronger between countries, indicating a high level of scientific cooperation on the subject. Figure 5 depicts on most productive journal in the context on oil spill response science.

Scholars can better grasp the academic preferences of each journal in the subject of oil spill by conducting research on high-quality journals, and thus be more focused when subscription to journals and publishing articles. As a result, we calculated the number of articles in the primary journals for oil spill response science research and ordered the top 10 journals by the number of articles published, as shown in Figure 5. A total of 107 journals, which are focused on a minimum number of five publications, were analyzed according to the number of articles. "Marine Pollution Bulletin" (n= 275, 10.91%), "Chemosphere" (n= 77, 2.94%), and "Environmental Pollution" (n= 75, 2.87%) were the most productive journals. The evaluated journals' WoS category was largely environmental science, and the 5-year impact factor weighted score was estimated as 6.645, implying that the selected articles were subjected to careful and stringent quality control by editors and peer reviewers. Table 1 summarizes the most cited articles related to oil spill response science between 2000 to 2022.

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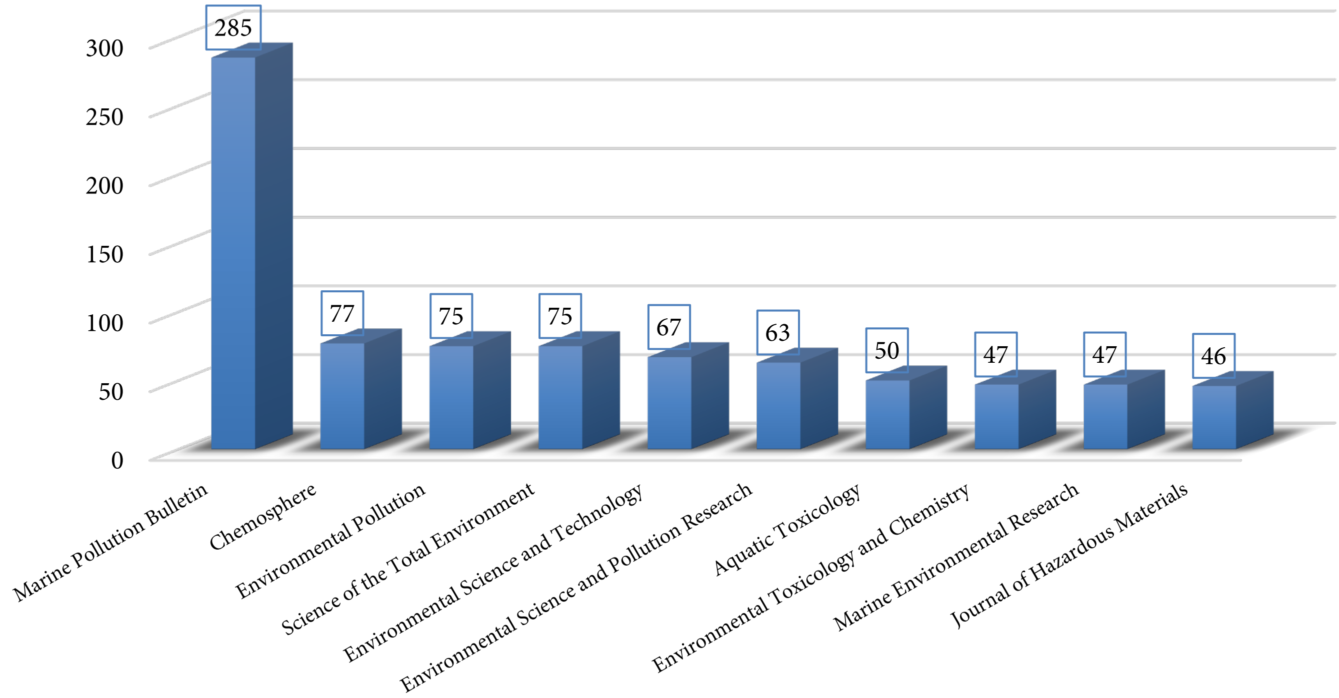

Figure 5. Most influential journal of oil spill response science

The most cited publications are also the most prominent in the subject of research. The first is by Xue et al. (2011) (1225 total citations), who presenting a proposal of materials that can be used in an innovative oil pollution response technique. The second is by Yuan et al. (2008) (883 total citations) carried out an experimental study presenting a self-assembly method to absorb oils up to 20 times, forming thermally stable, self-contained nanowire membranes in oil spill response. The third article is by Korhonen et al. (2011) (587 total citations) develop a product that is reusable, recyclable and has a high absorbent oil capacity to control oil spills. Figure 6 shows network mapping of co-authorship author analysis related to oil spill response studies.

To better understand the structure of scholarly contributions in this discipline, co-authorship and citation analyses were used. The examination of co-authorship analysis based on authors was limited to articles having a maximum of 25 authors per document, yielding a total of 10024 researchers. This number was subsequently decreased to 188 by only including authors who have published at least five times. Strongly collaborative authors from cluster 1 (20 authors, red) include Antonietta Quigg (Professor of Marine Biology, Texas A&M University, USA) and Samantha Joye (Professor of Oceanography, University of Georgia, USA). In cluster 2 (12 authors, blue), the most productive author includes Lee Kenneth (National Senior Science Advisor, Oil Spill Research, Preparedness, and Response for Fisheries and Oceans, Canada). In cluster 3 (11 authors, yellow) Lawrence S. Engel (UNC Gillings School of Global Public Health, USA) and Richard K. Kwok (National Institute of Environmental Health Sciences, U.S.A.) emerge as strong contributors. The striking detail in the co-author analysis is the researchers' work on the topics of oil pollution intervention after the BP Deep water horizon accident and its impact on human health. Figure 7 depicts co-word occurrence network analysis focusing on keywords that can be used to determine the state-of-the-art.

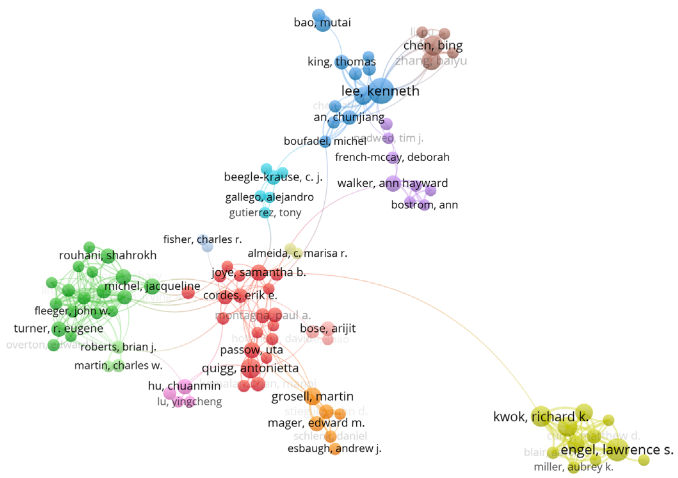

Figure 6. Oil spill response studies network map of co-authorship authors analysis

Table 1. Most cited articles related to oil spill response between 2000 to 2022

ID	Authors and Publication Year	Source Title	Total Citations	Average Per Year	Scientific Contribution	Aim of the Study	Methodology
1	Xue et al. (2011)	Advanced Materials	1225	102.08	To provide unique oil/water separation materials that have a high separation capacity, are resistant to oil fouling, and are completely recyclable.	To create an oil spill response material produced from recyclable, economical and environmentally friendly materials with high oil separation capacity	The theoretical and experimental analysis
2	Yuan et al. (2008)	Nature Nanotechnology	883	58.87	To propose an innovative material that could be favorable in the elimination of organics, notably in the field of environmental cleanup.	To present a self-assembly method for absorbing oils up to 20 times, forming thermally stable, self-contained nanowire membranes in oil spill response.	The theoretical and experimental analysis
3	Korhonen et al. (2011)	ACS Applied Materials & Interfaces	587	48.92	To pave the way for nanocellulose-based oil absorbents with long-term applications.	To develop a product that is reusable, recyclable or has a high absorbent oil capacity	Experimental analysis
4	Kujawinski et al. (2011)	Environmental Science and Technology	582	48.5	To offer essential geochemical parameters for future toxicological assessments and provide important restrictions on proper modeling of the deep-water dispersion	To perform experimental synthesis of environmental fate of dispersant on deep-water applications	Sample analysis and using ultrahigh resolution mass spectrometry and liquid chromatography
5	Boopathy (2000)	Bioresource Technology	507	22.04	To reveal all the factors affecting the bioremediation technique and determine the restrictions of application	To determine the different issues that limit the application of bioremediation technology, including various factors.	Review
6	Choi et al. (2011)	ACS Applied Materials & Interfaces	468	39	Ease of use, thanks to processes that are recyclable and have a simple preparation procedure	To create an innovative sponge that is recyclable, environmentally friendly and has a high oil absorption capacity	Experimental analysis
7	Atlas & Hazen (2011)	Nature Nanotechnology	459	38.25	Contribution to advanced molecular techniques to characterize microbial communities in oil pollution bioremediation technique	To offer a series lesson on the role of microbial biodegradation in determining the fate of the spill about two major oil spills	Review
8	Ge et al. (2017)	Environmental Science and Technology	388	64.67	A Joule-heated sorbent design has been developed for the first time to achieve high-speed cleanup of viscous crude-oil spills.	To propose an innovative sponge that reduces the viscosity grade of crude oil products	Experimental analysis and Optimization of efficiency
9	Ge et al. (2016)	Advanced Materials	354	50.57	To make implications for the production of next-generation oil absorbent materials, the production of oil absorbers, and the recycling of both oils and absorbent materials	To define the processes of manufacture, design, modification along with additional features that need to be developed for petroleum sorbents	Comparison between oil sorbents on technical, design and modification process
10	Singer et al. (2000)	Marine Pollution Bulletin	297	12.91	To standardize the preparation, analytical measurement and exposure method of the toxicity test environment used in the oil pollution response that can be used by all researchers.	To compare toxicity prediction tests that standardize both biological and analytical methods and provide a reliable and consistent database	Comparison between toxicology testing

Oil spill(s), deep water horizon, crude oil, bioremediation, Gulf of Mexico, biodegradation, dispersant, biomarkers, and polycyclic aromatic hydrocarbons were the ten words with the largest number of co-occurrences with repeating at least five keywords in general. These words are a strong indicator that they represent major conceptual and methodological structures in the literature on oil spill response. The greatest set of common word networks covering all years are correlated with phrases related to oil pollution response systems and the world's worst environmental disasters as can be seen in Figure 7. Oil spill response studies network map of co-cited references is shown in Figure 8.

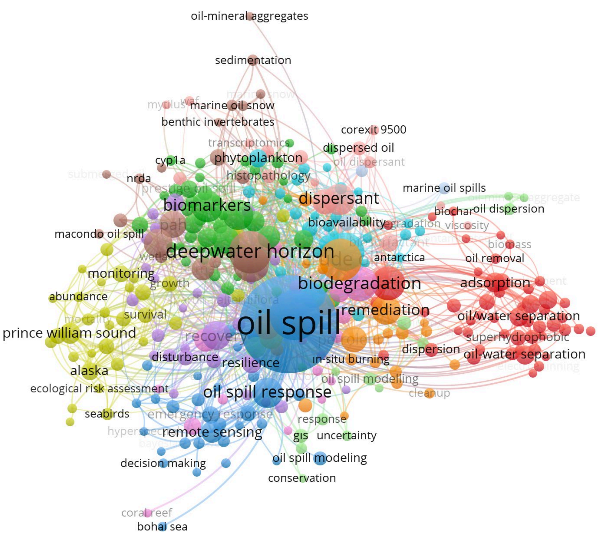

Figure 7. Network map of keywords related to oil spill response studies during 2000–2021

Co-citation, which is presented in science by Henry Small, indicates that two articles were cited jointly in the references of a third publication (Tan et al., 2021). A connecting line between two references indicates that two articles from separate journals are mentioned in the same article, and the strength of the connecting line indicates the degree of co-citation between the two articles (Herrera-Franco et al., 2021). Figure 8 depicts the co-citation networks of references as a visual representation of these articles, with red, green, yellow, and blue indicating the four primary clusters of co-cited articles and associated themes. Co-citation analysis of the 330 most relevant references with at least 20 citations from 2000 to 2022 revealed six clusters. The most prolific articles in Cluster 1 (red color), consisted of 92 articles, were Hazen et al. (2010) based on citations, and strength of link. In the reviewed article, a comprehensive study was carried out on the biological impacts of oil and its effect on indigenous oil-degrading bacteria after the Deep-water horizon blowout accident. Cluster 2 (green color) comprised of 89 publications that primarily used an environmental toxicology approach. The most cited article in Cluster 2 was by Singer et al. (2000), who standardize the preparation, analytical measurement and exposure method of the toxicity test environment used in the oil pollution response that can be used by all researchers. The other most prolific reference is Adebajo et al. (2003) in the Cluster 3 (Blue color). The synthesis and absorbing capabilities of a wide range of porous sorbent materials that have been researched for use in the removal of organics, notably in the area of oil spill cleanup, are reviewed in this paper.

Our research examines the evolution of research trends and recent theoretical dynamics from many perspectives and makes contributions in a variety of ways. First of all, the study makes an exploratory analysis to trace the highly cited research publications in oil spill response and summarizes the leading authors, revealed aim, methodology, and scientific contributions of literature related to oil spill response science. Second, this study makes a methodological contribution to the field of oil spill response science by presenting a bibliometric mapping that enables for the evaluation of scientific presentation as well as its visual analytics using three bibliometric mapping techniques (co-occurrence keyword analysis, author co-citations analysis, and co-authorship analysis), allowing for a better understanding of the field's structure and development.

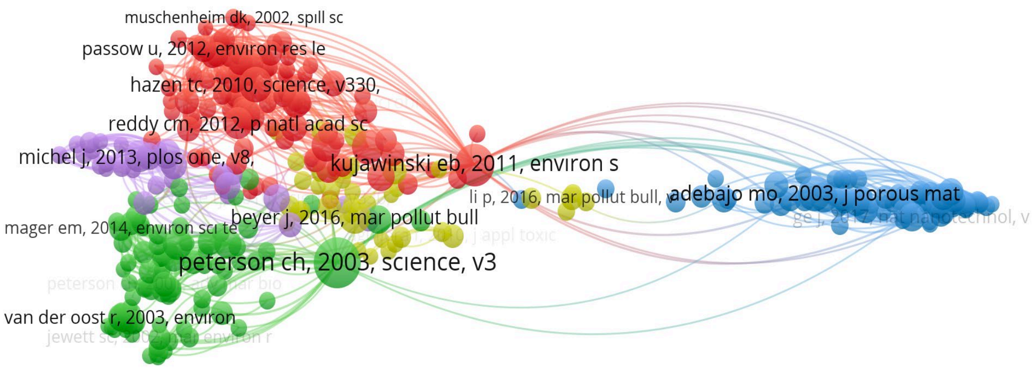

Figure 8. Oil spill response studies network map of co-cited references

The 2611 publications and research that have been mapped by imposing a minimum limit of 5 documents produced by 188 authors and their study presented in 107 journals, from universities and research centers in 58 countries, demonstrate growing interest in the topic. Xue et al. (2011) (who studied chemistry and molecular science) is the most mentioned article in these contributions (see Table 1), followed by Yuan et al. (2008). Both authors have made major contributions to the field of oil spill response science by conducting an experimental analysis and proposing a new and recyclable material for use in oil spill response. The usage of bibliometric maps creates a visual history of the research topic that is both interesting and informative. These maps enable us to draw the following conclusions:

- Keyword co-occurrence analysis indicated existing subjects as well as substantial improvements in the intellectual structure of oil spill response, as well as the stability and strength of other subjects (see Figure 7). Over an 87-day period, the Deepwater Horizon oil catastrophe dumped 134 million gallons of oil into the Gulf of Mexico, contaminating 1,300 miles of shoreline across five states. According to scientists, the Deepwater Horizon oil spill killed thousands of marine species and sea turtles while poisoning their ecosystems. It shows that the Deepwater Horizon catastrophe is regularly replicated in word analysis, and that the intervention approaches created as a result of accident analyses contribute significantly to this science. When the study development in Figure 2 is analyzed, it reveals the significance of the accident that occurred in 2010 in relation to our research topic.
- The most problematic topic is controlling and cleaning up marine oil spills, because it is difficult to retrieve all of the oil that has been spilled into the water. Physical, chemical, thermal, and biological cleanup approaches are current response techniques used (Dave & Ghaly, 2011). Many oil spills have already been treated with bioremediation, notably the one in Prince William Sound (which is the second most considerable accident frequently repeated in analyzes see in Figure 7). The bioremediation technique emerges as the leading technique when considering the materials developed in the studies as well as other clean-up techniques. This method of accelerating oil degradation by augmenting bacteria and nutrients to oil spills has a lower environmental impact than other recovery methods (Khalid et al., 2021). This will make communities more resistant and help them recover sooner after a spill.
- We identified the United States, China, Canada, and Spain to be the top four countries in terms of the number of publications, funding, and number of authors. The co-authorship examination of countries determined that China, the United States, Canada, and Italy were at the core of international cooperation, demonstrating this pattern (see Figure 4). When looking at the ITOPF (2021) study, it was discovered that oil pollution accidents, which have resulted in major environmental disasters around the world, occur in and around these four countries, and that research institutes are making progress on these concerns.
- Environmental Sciences, Marine Freshwater Biology, and Engineering were determined as the most prominent subject categories of knowledge in terms of the number of publications. The number of articles in each category reveals this, indicating that the field of oil spill response is likely relevant in terms of the themes addressed by the frequency of publications. Furthermore, when the categories of the most productive journals (see Figure 5) in Web of Science are studied, it is clear that the journals with the highest impact factor in the field of environmental science are those ranked by Journal Citation Indicator by Clarivate, Thomson Reuters.

This was the first attempt to map the oil spill response field in a methodical way. However, there are certain limits to our bibliometric analysis to consider. For instance, we only used one database (Web of Science) to assess impact, and we only used citations to do so. As a result, impact factors limited this approach, and it did not include all scientific publications. We analyzed peer-reviewed research articles, and pertinent review articles written in the English language in the sample to ensure homogeneity. Other publications, such as conference proceedings, book chapters, and reports could be useful in the future. We established a minimum threshold level between 2000 and 2022 because it is a new and emerging subject, and clusters were formed based on that threshold; this is a restrictions of this bibliometric mapping analysis because some potentially intriguing articles may have been removed. To summarize, the field of oil spills has expanded significantly, but more multidisciplinary research is required to completely investigate the numerous topics. Importantly, the study of response strategies needs more attention and emphasis in order to lead future research in this area. Further research is recommended in light of longer-term data and the diverse WoS categories found in oil spill science, which may be visualized using a variety of bibliometric visualization applications.

Compliance With Ethical Standards

Authors' Contributions

MB: Conceptualization, Software, Resources, Investigation, Methodology, Visualization, Formal analysis, Writing – review and editing

BK: Conceptualization, Writing – review and editing, Resources, Supervision, Investigation, Visualization

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Adebajo, M. O., Frost, R. L., Kloprogge, J. T., Carmody, O., & Kokot, S. (2003). Porous materials for oil spill cleanup: a review of synthesis and absorbing properties. *Journal of Porous Materials,* 10(3), 159-170. https://doi.org/10.1023/A:1027484117065

Alves, T. M., Kokinou, E., Zodiatis, G., Lardner, R., Panagiotakis, C., & Radhakrishnan, H. (2015). Modelling of oil spills in confined maritime basins: The case for early response in the Eastern Mediterranean Sea. *Environmental Pollution*, *206*, 390-399. https://doi.org/10.1016/j.envpol.2015.07.042

Atlas, R. M., & Hazen, T. C. (2011). Oil biodegradation and bioremediation: A tale of the two worst spills in US history. *Environmental Science and Technology*, *45*(16), 6709–6715. https://doi.org/10.1021/es2013227

Bonvicini, S., Bernardini, G., Scarponi, G. E., Cassina, L., Collina, A., & Cozzani, V. (2022). A methodology for response gap analysis in offshore oil spill emergency management. *Marine Pollution Bulletin*, *174*, 113272. https://doi.org/10.1016/j.marpolbul.2021.113272

Boopathy, R. (2000). Factors limiting bioremediation technologies. *Bioresource Technology*, *74*(1), 63-67. https://doi.org/10.1016/S0960-8524(99)00144-3

Bucelli, M., Landucci, G., Haugen, S., Paltrinieri, N., & Cozzani, V. (2018). Assessment of safety barriers for the prevention of cascading events in oil and gas offshore installations operating in harsh environment. *Ocean Engineering*, *158*, 171–185. https://doi.org/10.1016/j.oceaneng.2018.02.046

Chang, S. E., Stone, J., Demes, K., & Piscitelli, M. (2014). Consequences of oil spills: a review and framework for informing planning. *Ecology and Society*, *19*(2), 26. https://doi.org/10.5751/ES-06406-190226

Chen, D., Liu, Z., Luo, Z., Webber, M., & Chen, J. (2016). Bibliometric and visualized analysis of emergy research. *Ecological Engineering*, *90*, 285-293. https://doi.org/10.1016/j.ecoleng.2016.01.026

Chen, J., Di, Z., Shi, J., Shu, Y., Wan, Z., Song, L., & Zhang, W. (2020). Marine oil spill pollution causes and governance: A case study of Sanchi Tanker collision and explosion. *Journal of Cleaner Production*, *273*, 122978. https://doi.org/10.1016/j.jclepro.2020.122978

Chen, J., Zhang, W., Wan, Z., Li, S., Huang, T., & Fei, Y. (2019). Oil spills from global tankers: Status review and future governance. *Journal of Cleaner Production*, *227*, 20-32. https://doi.org/10.1016/j.jclepro.2019.04.020

Choi, S. J., Kwon, T. H., Im, H., Moon, D. I., Baek, D. J., Seol, M. -L., Duarte, J. P., & Choi, Y. -K. (2011). A polydimethylsiloxane (PDMS) sponge for the selective absorption of oil from water. *ACS Applied Materials & Interfaces,* *3*(12), 4552-4556. https://doi.org/10.1021/am201352w

Dave, D., & Ghaly, A. E. (2011). Remediation technologies for marine oil spills: A critical review and comparative analysis. *American Journal of Environmental Sciences*, *7*(5), 423-440. https://doi.org/10.3844/ajessp.2011.423.440

Ge, J., Shi, L. -A., Wang, Y. -C., Zhao, H. Y., Yao, H. -B., Zhu, Y. -B., Zhang, Y., Zhu, H. -W., Wu, H. -A., & Yu, S. -H. (2017). Joule-heated graphene-wrapped sponge enables fast clean-up of viscous crude-oil spill. *Nature Nanotechnology,* *12*(5), 434-440. https://doi.org/10.1038/nnano.2017.33

Ge, J., Zhao, H. Y., Zhu, H. W., Huang, J., Shi, L. A., & Yu, S. H. (2016). Advanced sorbents for oil-spill cleanup: recent advances and future perspectives. *Advanced Materials,* *28*(47), 10459-10490. https://doi.org/10.1002/adma.201601812

Gonçalves, M. C. P., Kieckbusch, T. G., Perna, R. F., Fujimoto, J. T., Morales, S. A. V., & Romanelli, J. P. (2019). Trends on enzyme immobilization researches based on bibliometric analysis. *Process Biochemistry*, *76*, 95-110. https://doi.org/10.1016/j.procbio.2018.09.016

Hazen, T. C., Dubinsky, E. A., DeSantis, T. Z., Andersen, G. L., Piceno, Y. M., Singh, N., Jansson, J. K., Probst, A., Borglin, S. E., Fortney, J. L., Stringfellow, W. T., Bill, M., Conrad, M. E., Tom, L. M., Chavarria, K. L., Alusi, T. R., Lamendella, R., Joyner, D. C., Spier, C., Baelum, J., … Mason, O. U. (2010). Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science*, 330(6001), 204-208. https://doi.org/10.1126/science.1195979

Herrera-Franco, G., Montalván-Burbano, N., Mora-Frank, C., & Moreno-Alcívar, L. (2021). Research in petroleum and environment: A bibliometric analysis in South America. *International Journal of Sustainable Development and Planning*, *16*, 1109-1116. https://doi.org/10.18280/ijsdp.160612

Hung, P. V., Kim, K. S., Tien, L. Q., & Cuong, N. M. (2018). Distribution of oil spill response capability through considering probable incident, environmental sensitivity, and geographical weather in Vietnamese waters. *Journal of International Maritime Safety, Environmental Affairs, and Shipping*, *2*(1), 31-41. https://doi.org/10.1080/25725084.2018.1511240

ITOPF (The International Tanker Owners Pollution Federation Limited). (2021). *Oil tanker spill statistics 2021*. Retrieved on February 11, 2022, from https://www.itopf.org/knowledge-resources/data-statistics/statistics/

Khalid, F. E., Lim, Z. S., Sabri, S., Gomez-Fuentes, C., Zulkharnain, A., & Ahmad, S. A. (2021). Bioremediation of diesel contaminated marine water by bacteria: A review and bibliometric analysis. *Journal of Marine Science and Engineering*, *9*(2), 155. https://doi.org/10.3390/jmse9020155

Korhonen, J. T., Kettunen, M., Ras, R. H., & Ikkala, O. (2011). Hydrophobic nanocellulose aerogels as floating, sustainable, reusable, and recyclable oil absorbents. *ACS Applied Materials & Interfaces*, *3*(6), 1813-1816. https://doi.org/10.1021/am200475b

Krohling, R. A., & Campanharo, V. C. (2011). Fuzzy TOPSIS for group decision making: A case study for accidents with oil spill in the sea. *Expert Systems with Applications*, *38*(4), 4190-4197. https://doi.org/10.1016/j.eswa.2010.09.081

Kujawinski, E. B., Kido Soule, M. C., Valentine, D. L., Boysen, A. K., Longnecker, K., & Redmond, M. C. (2011). Fate of dispersants associated with the Deepwater Horizon oil spill. *Environmental Science & Technology*, *45*(4), 1298-1306. https://doi.org/10.1021/es103838p

Li, P., Cai, Q., Lin, W., Chen, B., & Zhang, B. (2016). Offshore oil spill response practices and emerging challenges. *Marine Pollution Bulletin*, *110*(1), 6-27. https://doi.org/10.1016/j.marpolbul.2016.06.020

Lim, Z. S., Wong, R. R., Wong, C. Y., Zulkharnain, A., Shaharuddin, N. A., & Ahmad, S. A. (2021). Bibliometric analysis of research on diesel pollution in Antarctica and a review on remediation techniques. *Applied Sciences*, 11(3), 1123. https://doi.org/10.3390/app11031123

Mohammadiun, S., Hu, G., Gharahbagh, A. A., Li, J., Hewage, K., & Sadiq, R. (2021). Intelligent computational techniques in marine oil spill management: A critical review. *Journal of Hazardous Materials*, *419*, 126425. https://doi.org/10.1016/j.jhazmat.2021.126425

Murphy, D., Gemmell, B., Vaccari, L., Li, C., Bacosa, H., Evans, M., Gemmell, C., Harvey, T., Jalali, M., & Niepa, T. H. (2016). An in-depth survey of the oil spill literature since 1968: Long term trends and changes since Deepwater Horizon. *Marine Pollution Bulletin*, *113*(1-2), 371-379. https://doi.org/10.1016/j.marpolbul.2016.10.028

Neves, A. A. S., Pinardi, N., Martins, F., Janeiro, J., Samaras, A., Zodiatis, G., & De Dominicis, M. (2015). Towards a common oil spill risk assessment framework–adapting ISO 31000 and addressing uncertainties. *Journal of Environmental Management*, *159*, 158-168. http://dx.doi.org/10.1016/j.jenvman.2015.04.044

Passos, A. C., Teixeira, M. G., Garcia, K. C., Cardoso, A. M., & Gomes, L. F. A. M. (2014). Using the TODIM-FSE method as a decision-making support methodology for oil spill response. *Computers & Operations Research*, *42*, 40-48. https://doi.org/10.1016/j.cor.2013.04.010

Pauna, V. H., Buonocore, E., Renzi, M., Russo, G. F., & Franzese, P. P. (2019). The issue of microplastics in marine ecosystems: A bibliometric network analysis. *Marine Pollution Bulletin*, *149*, 110612. https://doi.org/10.1016/j.marpolbul.2019.110612

Prendergast, D. P., & Gschwend, P. M. (2014). Assessing the performance and cost of oil spill remediation technologies. *Journal of Cleaner Production*, *78*, 233-242. https://doi.org/10.1016/j.jclepro.2014.04.054

Robbe, N., & Hengstermann, T. (2006). Remote sensing of marine oil spills from airborne platforms using multi-sensor systems. *Water Pollution VIII: Modelling,* *Monitoring and Management*, *1*, 347-355. https://doi.org/10.2495/WP060351

Singer, M. M., Aurand, D., Bragin, G. E., Clark, J. R., Coelho, G. M., Sowby, M. L., & Tjeerdema, R. S. (2000). Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Marine Pollution Bulletin, 40*(11), 1007-1016. https://doi.org/10.1016/S0025-326X(00)00045-X

Sirimanne, S. N., Hoffman, J., Juan, W., Asariotis, R., Assaf, M., Ayala, G., ... & Premti, A. (2019). Review of maritime transport 2019. *In United Nations Conference on Trade and Development*, Geneva, Switzerland.

Su, K., Song, Y., & Jiang, Y. (2014). Research on emergency response capacity of water pollution accident based on structural equation. *Journal of Wuhan University of Technology, 36*, 114-119. (In Chinese)

Sweileh, W. M., Al-Jabi, S. W., AbuTaha, A. S., Zyoud, S. H., Anayah, F. M. A., & Sawalha, A. F. (2017). Bibliometric analysis of worldwide scientific literature in mobile - health: 2006–2016. *BMC Medical Informatics and Decision Making, 17*(1), 72. https://doi.org/10.1186/s12911-017-0476-7

Tan, H., Li, J., He, M., Li, J., Zhi, D., Qin, F., & Zhang, C. (2021). Global evolution of research on green energy and environmental technologies: A bibliometric study. *Journal of Environmental Management*, *297*, 113382. https://doi.org/10.1016/j.jenvman.2021.113382

Van Eck, N. J., & Waltman, L. (2013). *VOSviewer manual. 1*(1), 1-53. Leiden.

Vasconcelos, R. N., Lima, A. T. C., Lentini, C. A., Miranda, G. V., Mendonça, L. F., Silva, M. A., Cambui, E. C. B., Lopes, J. M., & Porsani, M. J. (2020). Oil spill detection and mapping: A 50-year bibliometric analysis. *Remote Sensing, 12*(21), 3647. https://doi.org/10.3390/rs12213647

Wang, J., Zhou, Y., Zhuang, L., Shi, L., & Zhang, S. (2022). Study on the critical factors and hot spots of crude oil tanker accidents. *Ocean & Coastal Management*, *217*, 106010. https://doi.org/10.1016/j.ocecoaman.2021.106010

Wong, S. L., Nyakuma, B. B., Wong, K. Y., Lee, C. T., Lee, T. H., & Lee, C. H. (2020). Microplastics and nanoplastics in global food webs: A bibliometric analysis (2009–2019). *Marine Pollution Bulletin*, *158*, 111432. https://doi.org/10.1016/j.marpolbul.2020.111432

Xue, Z., Wang, S., Lin, L., Chen, L., Liu, M., Feng, L., & Jiang, L. (2011). A novel superhydrophilic and underwater superoleophobic hydrogel-coated mesh for oil/water separation. *Advanced Materials*, *23*(37), 4270-4273. https://doi.org/10.1002/adma.201102616

Yang, Z., Chen, Z., Lee, K., Owens, E., Boufadel, M. C., An, C., & Taylor, E. (2021). Decision support tools for oil spill response (OSR-DSTs): Approaches, challenges, and future research perspectives. *Marine Pollution Bulletin*, *167*, 112313. https://doi.org/10.1016/j.marpolbul.2021.112313

Yuan, J., Liu, X., Akbulut, O., Hu, J., Suib, S. L., Kong, J., & Stellacci, F. (2008). Superwetting nanowire membranes for selective absorption. *Nature Nanotechnology*, *3*(6), 332-336. https://doi.org/10.1038/nnano.2008.136

Zou, X., Yue, W. L., & Le Vu, H. (2018). Visualization and analysis of mapping knowledge domain of road safety studies. *Accident Analysis & Prevention*, *118*, 131-145. https://doi.org/10.1016/j.aap.2018.06.010

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RESEARCH ARTICLE

Effect of abrupt salinity change in the survival of Asian green mussel *Perna viridis* (Linnaeus, 1758) spats

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ABSTRACT

Salinity is one of the key environmental factors that affects the growth and survival of marine organisms including mussels. Five different salinity levels (40, 30, 20, 10 and 5 ppt) were used to test the effect of abrupt salinity change in the survival of hatchery-produced spats of Asian green mussel *Perna viridis* (Linnaeus, 1758). Spats were stocked with a density of 30 individuals per 6-L tank. Salinity manipulation was conducted after 2 days from the date of stocking. Based on the results, abrupt change and prolonged exposure to lower salinities particularly of 5 and 10 ppt (up to 5 days) are detrimental to *P. viridis* spats. The critical time for *P. viridis* spats wherein they could seclude themselves from the persistent lower salinities is 28 hours from its exposure. After which, mortality could be high at about 50% and will continue in the succeeding days if low salinity persists. Critically, no single spat can survive until the 4th day of continuous exposure to very low salinity of 5 ppt. Nevertheless, surviving individuals could still recover if salinity will return to optimum levels. Additionally, spats can readily adjust to abrupt change up to 10 ppt from the optimum salinity level as seen in the high survival in 20 and 40 ppt.

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Introduction

Asian green mussel *Perna viridis* (Linnaeus, 1758) is a marine bivalve species belonging to family Mytilidae. The species is widely distributed along the coasts of India in the Indo-Pacific and in Southeast Asia (Rajagopal et al., 2006), and has been introduced in the coastal areas of the western Hemisphere including Venezuela, Trinidad, Jamaica and Florida in the United States (Ingrao et al., 2001). Its habitats range from oceanic to estuarine waters (McFarland et al., 2013), usually at a depth of less than 10 m (Iqbal et al., 2018). Aside from its ecological importance, the species is an important seafood resource and is extensively cultivated for commercial purpose especially in Southeast Asia. This species is now considered a cheap source of protein with high nutritional values and known for its palatable taste (Taib et al., 2016).

Salinity is one of the key environmental factors that affects the growth and survival of marine organisms, particularly in shallower waters where sessile organisms such as mussels occur (Wang et al., 2011; Wu et al., 2018). In the case of *P. viridis,* the optimal salinity ranged from 27 – 35 ppt (Aypa, 1990; Rajagopal et al., 2006). However, in estuarine and coastal systems where these mussels typically occur, salinity fluctuations are considerable. During freshwater influx and heavy rains which decrease the water salinity in these areas, mussel growth and survival are negatively affected (Cheung, 1991; Soon & Ransangan, 2016; Wang et al., 2012). In the Zhoushan islands sea area, mussels often experience short-term salinity and pH fluctuations from 15-35 ppt and 7.3–8.1, respectively, leading to increased mortality of the organisms during wet season (Wu et al., 2018).

In the Philippines, *P. viridis* is widely cultured for commercial purpose (Aypa, 1990). The aquaculture production of green mussel in 2018 reached to 26,302.77 mt valued at 9.8 M USD (PSA, 2019). However, the production of this species in the recent years appeared to be declining (Duncan et al., 2009), and this decline was linked to the decreasing spatfall in the wild (Mero et al., 2019). The supply of seed called spats for the culture of *P. viridis* is dependent from the natural environment in which seed stock is unstable. Sustainable seed supply of cultured mussel species such as *P. viridis* is a crucial component for the success of the shellfish industry. With the aim to augment the seed supply and lessen the reliance of mussel farmers from getting their seeds in the wild, the Philippine government invested for a mussel hatchery in 2014 (Mero et al., 2019).

The natural habitat where *P. viridis* is grown is exposed to constant environmental changes, hence, it is important to know how the hatchery-produced spat may be affected particularly by salinity fluctuations. This study aims to determine the survival of hatchery-produced *P. viridis* spats subjected to abrupt changes in salinity, and determine how long these spats could withstand salinity stress.

Materials and Methods

Experimental Animal and Study site

This study was conducted in September 2019 in the Green Mussel Hatchery and Nursery (GMHN) of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo. The *P. viridis* spats used in the experiment were produced in the same hatchery. These spats were one and a half months old with sizes ranging from 5-9 mm shell length (widest part from the posterior to the anterior part of the shell) measured using a plastic Vernier caliper.

Experimental Design

The effect of abrupt salinity change in the survival of *P. viridis* spats was tested using five salinity levels (40, 30, 20, 10 and 5 ppt). Each salinity level served as treatment with three replicates. The 30 ppt treatment being within the optimum salinity range for the species (Aypa, 1990) served as control, so that its salinity was not changed the entire experimental period which lasted for 10 days.

This experiment was conducted in 6-L plastic rectangular tanks. Each tank contained 4 L of water as its final volume having a specific salinity (i.e., 40, 30, 20, 10 and 5 ppt). Nevertheless, all treatments had an initial salinity of 30 ppt. The spats were stocked with a density of 30 individuals per tank. Salinity manipulation (e.g., from 30 ppt to 40 ppt) was conducted after 2 days from the date of stocking.

Following the methods of Ponce-Palafox et al. (1997) and Su et al. (2010), the salinity was increased (for 40 ppt) by progressively adding salt solution using sea salt (bought from the local market) to a separate water stock tank until the desired salinity was obtained, and was left aerated. On the other hand, salinity was decreased (20, 10 and 5 ppt) by adding an aerated tap water to the tanks. The salinities of the stock tanks for seawater as well as the experimental tanks were checked prior to the start of the experiment and in the morning of the succeeding days using a handheld refractometer (ATAGO). The addition of either salt solution or freshwater to the experimental tanks to abruptly change the salinity was done gradually within 30 minutes (only during the onset of the salinity change). The time frame follows what was recorded by Casila et al. (2017) in Shakuji river estuary, Japan where salinity fluctuated within 30 minutes after freshwater intrusion. Respective salinities (40, 30, 20, 10 and 5 ppt) were maintained for 5 days. On the 6th day, salinities of all treatments except for the control were abruptly returned to 30 ppt and maintained until the end of the experiment to check if the spats could survive or recover after being subjected to abrupt salinity change (lower or higher) and abrupt return to optimal condition. The water stock tanks and the experimental tanks were kept aerated through the centralized aeration system of GMHN until the end of the experiment.

The spats were fed with algae, *Isochrysis galbana* and *Tetraselmis tetrathele* which were given twice daily at 8:00 -9:00 in the morning and 4:00-5:00 in the afternoon. The daily food ration was calculated based on the formula provided by Helm & Bourne (2004) as follow (Eq. 1):

$$V = \frac{S \times 0.4}{W \times C} \quad (1)$$

where V is the volume of algae in liters to be fed, S is the live weight of spat in mg, W is the weight of the required algal cells, C is the algal density (cells per μL), and 0.4 refers to the ration as dry weight of the algae in mg per mg live weight of the spat. The algae were sourced from the Southeast Asian Fisheries Development Center Aquaculture Department and sub-cultured in GMHN following the methods of de la Peña & Franco (2013). With the use of UV filtered seawater, algae were batch cultured in 800 mL glass bottles and to 10 L plastic carboys. All cultures were enriched with Conwy, and were subjected to 24 hours constant illumination with continuous aeration. Water exchange for all experimental tanks was done every after 2 days.

Data Collection and Analysis

Monitoring though direct observation and with the aid of a video recorder was conducted in 1, 4, 6, 8, 12, 24 and 28 hours after salinity had been changed. For the succeeding days, monitoring was conducted once daily (morning), that is before feeding to check for mortalities. Dead individuals if present were removed immediately to prevent degradation of water quality, and were accounted for later analysis. An individual was considered dead if it remained gaping but did not respond within 15 seconds when gently poked by a needle following the method of Segnini de Bravo et al. (1998), Yuan et al. (2016) and Binzer et al. (2018).

Survival rate was calculated by the final number of surviving spats divided by the initial number of spats (stocking density), multiplied by 100 following Vu & Huynh (2020). Gaping percentage was calculated as the number of individuals with valves open divided by the number of surviving individuals during the latest monitoring multiplied by 100. These data are the ones used in the statistical analysis. Gaping as used in this paper referred to the spat which gapes and responds to stimuli particularly to the gentle poke using a needle during a specific monitoring period.

One-way Analysis of Variance (ANOVA) was used to determine significant differences in the survival and gaping among treatments and Tukey's Test for post-hoc. All analyses were done in JASP 0.16.

Results and Discussion

Green mussel spats used in this experiment generally showed a wide range of salinity tolerance similar to other previous reports (Segnini de Bravo et al., 1998; Segnini de Bravo, 2003; Rowchai, 2004; McFarland et al., 2014). The study of McFarland et al. (2014) showed that *P. viridis* were able to tolerate low salinities up to 6 ppt when subjected to gradual salinity change. However, acute salinity changes of 15 ppt or more resulted to low survival. Rowchai (2004) recorded only 26.25% mortality from 15 ppt salinity treatment. Thus, the author suggested to set the lower salinity threshold for *P. viridis* at 15 ppt. Segnini de Bravo et al. (1998) reported a higher salinity tolerance of *P. viridis* under a gradual change of salinity level at 2 ppt every 2 days. The low and high lethal salinities for the species were recorded at 0 and 64 ppt, respectively. The report of Segnini de Bravo (2003) also proven the adaptability of *P. viridis* to salinity fluctuations. The species exhibited physiological compensation to increase (up to 45 ppt) or decrease (up to 15 ppt) in salinity under a change rate of 1 ppt per day.

Survival rate and response in terms of valve opening in different salinities are shown in Figure 1. Generally, survival rate is lower in reduced salinities (i.e., 10 and 5 ppt) and higher in higher salinities (i.e., 40, 30 and 20 ppt). From the time of salinity change, no single individual was observed having its valves open until 24 hours in 5 ppt and 8 hours in 10 ppt. This suggests that the spats from these treatments are negatively affected by such low salinity, prompting them to seclude

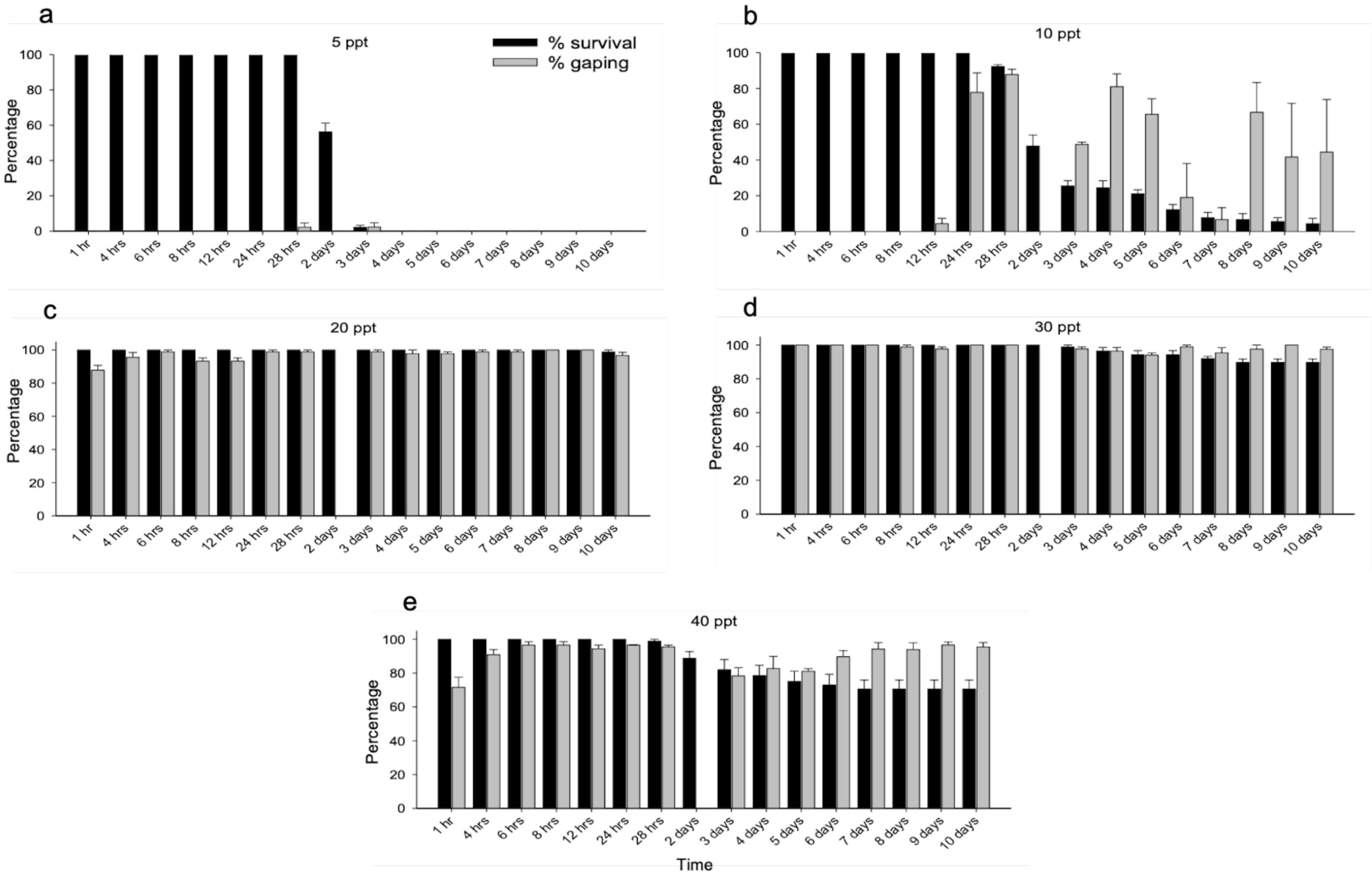

Figure 1. Survival and gaping percentage of *P. viridis* spats (mean±SEM) per salinity treatment. For the first 5 days, salinity was abruptly changed to: a) 5 ppt, b) 10 ppt, c) 20 ppt and e) 40 ppt (30 ppt served as control, thus salinity was not changed). On the 6th day, salinity of all the 4 treatments were abruptly returned to 30 ppt.

themselves by tightly closing their valves. During monitoring on the 28th hour, about 2% of the spats from 5 ppt (Figure 1a) were observed already gaping, and this very low percentage remained until all individuals died on the 4th day. This basically implies, that drastic decrease of salinity into very low level such as 5 ppt would lead to massive mortality of *P. viridis*. This may be true in the wild conditions especially during prolonged heavy rains wherein salinity could drop to certain low level. On the other hand, about 4% were gaping from 10 ppt during the 12th hour monitoring and increased to 77.78% in the 24th hour (Figure 1b). Mortalities started on the 28th hour and continued until only 21.11% remained surviving on the 5th day when salinity remained at 10 ppt. It seems that *P. viridis* could somehow tolerate 10 ppt salinity level but only for a short period of time. Long time exposures could also be detrimental to the organism. It also took about 12 hours adjustment before most could open their valves. When salinity was returned to 30 ppt, mortalities still continued until only 4.44% left surviving on the 10th day. In addition, not a single monitoring time, that spats from 10 ppt were seen 100% gaping. It seems that salinity adjustment ability of *P. viridis* is impaired when it was previously subjected to stressful low salinity such as 10 ppt.

For higher salinity treatments, high percentage of spats remained gaping despite salinity change. In 20 ppt (Figure 1c), 87.78% remained its valves open during abrupt salinity change. Gaping percentage for this treatment remained high varying from 93.33%–100% until the end of the experiment, and despite the salinity was abruptly returned to 30 ppt from 6th–10th day. No mortality occurred in this treatment except for one individual on the 10th day. This shows that 20 ppt is still optimum for the survival of *P. viridis* spats.

For the 30 ppt (Figure 1d), as expected, all spats remained gaping even after water change since the salinity was constant and within the optimum range for *P. viridis*. Though very little percentage (2.22% at most) were observed closed during monitoring periods. On the other hand, neglectable mortalities occurred within the 3rd to 8th day. For 40 ppt (Figure 1e), only 71.61% were observed gaping after salinity was increased. On the 4th hour, gaping percentage increased to 95.55%. High gaping percentage remained high varying from 94.37% to

96.59% until the 28th hour. However, gaping percentage dropped to 80% from the 3rd until 5th day. When the salinity was returned to 30 ppt on the 6th day, gaping increased to 89.67% and remained high. Survival became stable from the 7th to the 10th day, which may imply that the organisms were now adjusted to the present salinity condition.

Effect of Abrupt Salinity Change in the Survival and Valve Opening of P. viridis Spats

In this study, abrupt change of salinities at various levels significantly affected the valve opening and survival of *P. viridis*. On the 1st hour (Figure 2a), gaping percentage was highest in 20 and 30 ppt followed by 40 ppt, whilst individuals from 5 and 10 ppt were not able to open its valves. On the 4th hour (Figure 2b), gaping individuals remained highest in 20 and 30 ppt, while more individuals gaped in 40 ppt making its percentage comparable to 20 ppt, whilst still no individuals gaped in 5 and 10 ppt.

On the 6th, 8th,12th and 24th hour, percentage of gaping individuals did not vary among 20, 30 and 40 ppt, whilst still no gaping individuals were observed in 5 and 10 ppt, except a few from the later on the 12th hour. Similarly, McFarland et al. (2013) observed that *P. viridis* tended to close its valves at lower salinities of 10 and 15 ppt, this in spite of the fact the organisms used in their study were already adults with shell lengths ranging from 90-110 mm.

Stressful environmental condition such as salinities outside the optimum range of bivalves including *P. viridis* would trigger the organism to close its valves to seclude and protect itself from the external environment (McFarland et al., 2013). But this closure as defense mechanism especially when prolonged could lead to serious implications in the organism's being. Valve opening or gaping has a strong influence in many physiological processes in bivalve species (Nicastro et al., 2010). Bivalves have to open its valves for water filtration, feeding and respiration (Ballesta-Artero et al., 2017). Thus, valve closure results to cessation in feeding and gas exchange with the external environment. When the mussel stops feeding, it may use some stored energy which will eventually run out. On the other hand, since the organism can't exchange gas with the external environment, prolonged valve closure would lead to anoxic respiration. These could then lead to fatality of the organism. In the study of Comeau et al. (2018), it showed that raft-cultivated mussel *Mytilus galloprovincialis* gapes most of the time (97.5±1.3%) within the entire 10 days of monitoring on the gaping behavior of the organism. However, the reason of the occasional closures is not clear. It was also noted that valve closures are not synchronized among individuals, similar to *P. viridis* used in this study. Comeau et al. (2018) also noted that valve closure was physiologically regulated rather than environmentally mediated.

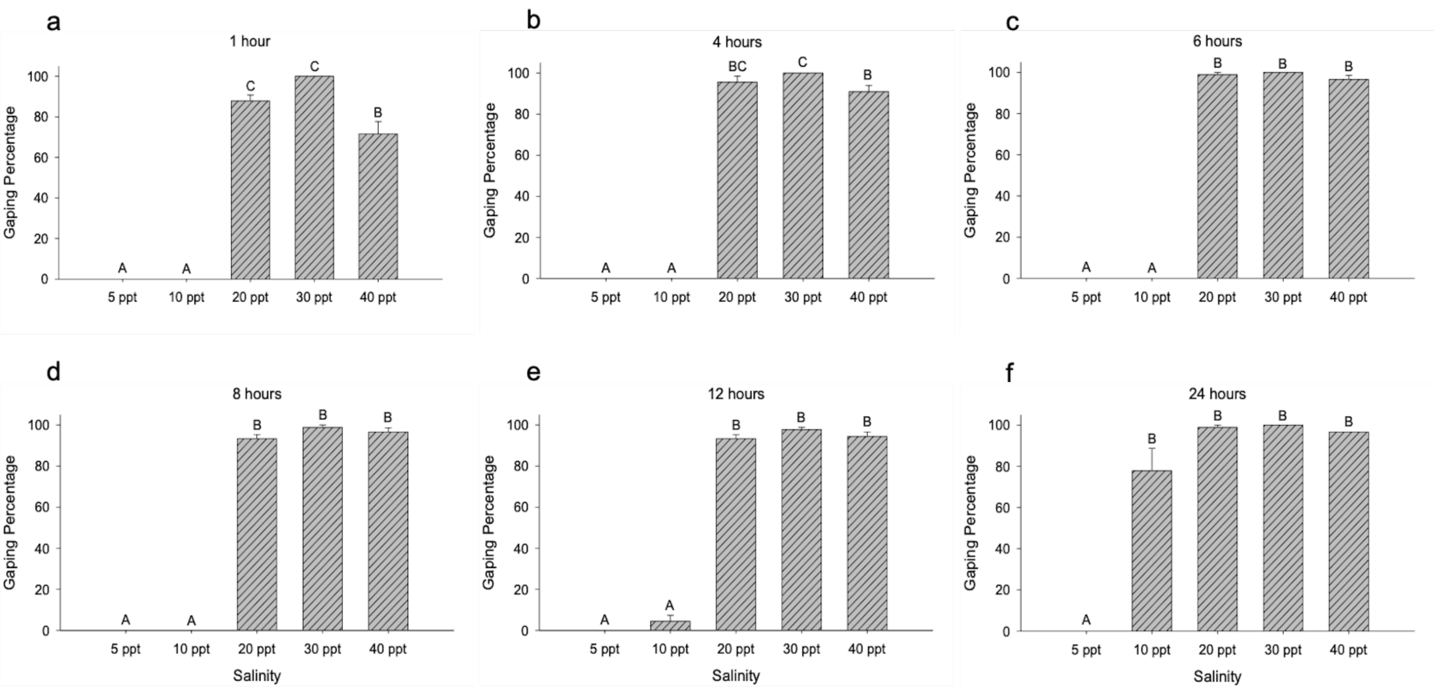

Figure 2. Gaping percentage of *P. viridis* spats (mean±SEM) at different salinity levels after: a) 1 hour, b) 4 hours, c) 6 hours, d) 8 hours, e) 12 hours and f) 24 hours, from the time that salinity was abruptly changed at different salinities, except 30 ppt which served as control. Means of different subscripts indicate a significant difference ($p<0.05$).

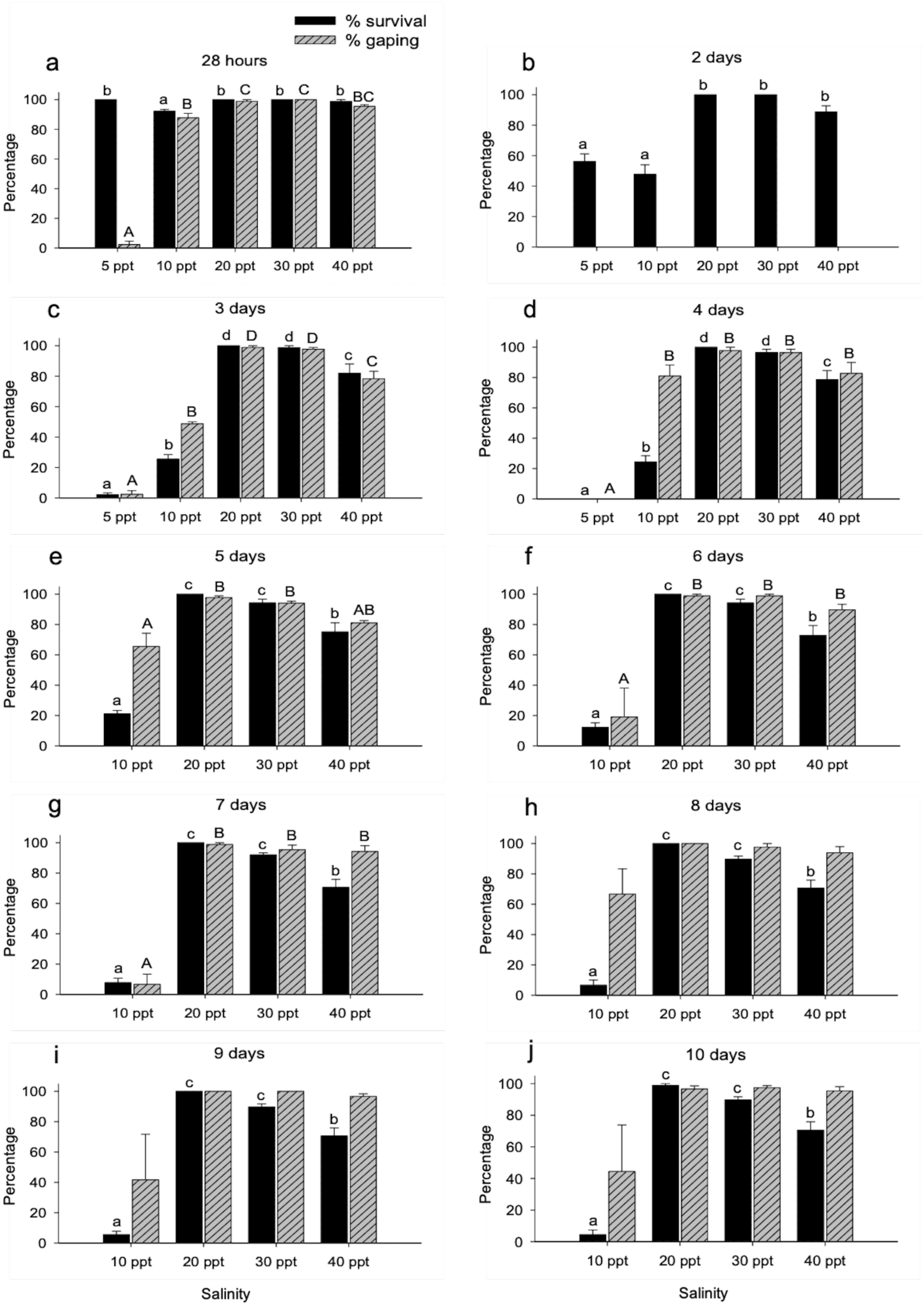

Figure 3. Survival and gaping percentage of *P. viridis* spats (mean±SEM) at different salinity levels after: a) 28 hours, b) 2 days, c) 3 days, d) 4 days, e) 5 days, f) 6 days, g) 7 days, h) 8 days, i) 9 days and j) 10 days. For the first 5 days, salinity was abruptly changed to: 1) 5 ppt, 2) 10 ppt, 3) 20 ppt and 4) 40 ppt (30 ppt served as control, salinity was not changed). On the 6th day, Salinity of the all 4 treatments were abruptly returned to 30 ppt. Means of different subscripts indicate a significant difference (p>0.05).

The valve closure may be induced by a full gut or some likely mechanism. On the other hand, this study noted that a gaping individual once closes its valves re-opens for a split to very few seconds particularly in the higher salinity levels (i.e., 20, 30 and 40 ppt).

Mortalities based on the indicator that valves of an individual are open but unresponsive to stimuli started on the 28th hour (Figure 3a), particularly at 10 ppt. Thus a 100% survival in the first 24 hours was assumed. On the 28th hour, some individuals died in 10 ppt so that its survival rate becomes significantly lower than the rest of the treatments (p<0.01). A significantly lower gaping percentage was also recorded in 10 ppt compared to the 3 higher salinities. On the other hand, a significantly few individuals from 5 ppt were observed gaping during this period. These few individuals may have forced themselves to open their valves as they are already starving or needing for gas exchange, but were not able to withstand such low salinity, thus reclosed and died subsequently. On the 2nd day (Figure 3b), mortalities from 5 and 10 ppt becomes conspicuous at about 50% giving a lower survival rate compared to the 3 higher salinities. From the 3rd until 10th day (Figure 3c- 3j), survival from 20 and 30 ppt remained significantly highest, followed by 40 ppt and 10 ppt. In terms of gaping percentage, this was highest in 20 and 30 ppt followed by 40 ppt, then 10 ppt and 5 ppt. On the 4th day (Figure 3d), gaping percentage in 5 ppt became zero (0) since all individuals were found dead on that day, whilst no significant difference (p>0.05) was found among higher salinities (10, 20, 30 and 40 ppt). The 5 ppt treatment was no longer included in the analysis from the 5th until the 10th day (Figure 3e- 3j) given that there was no more representative sample. These results are similar to the report of McFarland et al. (2014) where mussels that were immediately transferred from 30 ppt to 5 and 10 ppt salinity treatments were all dead within 13 days. Wider salinity difference even within a gradual change scheme also rendered significant mortality up to 100% in 3 ppt within 13 days and 47% mortality in 6 ppt within 28 days. Decreased byssal attachment and increased valve closure where also observed for *P. viridis* at 3 and 6 ppt. McFarland et al. (2013), reported that all individuals in lower salinities (10 and 15 ppt) remained either closed or dead after 120 hours. Rowchai (2004) also observed 100% mortality from 5 and 10 ppt within 72 hours. These mortalities are attributed to the persistent unfavorable water condition such as very low salinity that instigates prolonged valve closure and disables the ability of the organism to remain open long enough for feeding and sufficient gas exchange (McFarland et al., 2013). On the other hand, Segnini de Bravo et al. (1998) recorded a low mortality of *P. viridis* under gradual salinity change at 2 ppt every 2 days. The observed mortalities were only 2% and 4% from 4 ppt and 0 ppt salinity treatments, respectively.

On the 5th day (Figure 3e), gaping percentage was still significantly higher in higher salinities (20 and 30 ppt) except for 40 ppt which did not differ significantly with 10 ppt but comparable with 30 ppt. On the 6th until the 7th day (Figure 3f-3g), when salinities were abruptly returned to 30 ppt, gaping percentage were still significantly higher (p<0.01) among individuals from higher salinity treatments (20, 30 and 40 ppt) than 10 ppt. On the 8th until the 10th day (Figure 3h- 3j), gaping percentages were no longer significantly different among treatments (p>0.05), noting that there were only less than 10% surviving individuals in 10 ppt. Survival and gaping activity generally stabilized in the 7th to the 10th day (Figure 3g- 3j), wherein salinities were already returned to 30 ppt. McFarland et al. (2013) noted that gaping individuals are significantly higher in higher salinities of 25 and 35 ppt. McFarland et al. (2014) observed a high survival of *P. viridis* (more than 85%) despite drastic change provided that the salinity difference is within 10 ppt (i.e., from 30 ppt to 20, 25 and 35 ppt). Given a gradual salinity change, *P. viridis* was more tolerant with 97% survival at salinity treatments of 9 ppt and higher. Comparably, 100% survival at 20 and 25 ppt was obtained in the experiment of Rowchai (2004). In this study, hatchery produced spats can readily adjust to abrupt salinity changes up to 10 ppt.

Conclusion

Abrupt change and prolonged exposure to lower salinities particularly, 5 and 10 ppt are detrimental to *P. viridis* spats. The critical time for *P. viridis* spats wherein they could seclude themselves for the persistent lower salinities is 28 hours from its exposure. After which, mortality could be high at about 50% and will continue in the succeeding days if low salinity persists. On the other hand, if salinity will return to optimum levels, surviving individuals may recover. This study shows that hatchery-produced spat has also a wide range salinity tolerance wherein it may able to survive salinities outside its optimum range as low as 10 ppt and as high as 40 ppt, though a very high mortality may occur in the former. Additionally, hatchery produced spats can readily adjust to abrupt salinity change of up to 10 ppt (e.g., from 30 ppt to 20 ppt and 40 ppt).

The results of this study show the resilience of hatchery produced spats from drastic salinity fluctuations that typically happens in the natural environment. This study also gives idea on the salinity threshold of hatchery-produced spats that will serve as guide especially in the selection of site where this mussel species is to be transplanted for grow-out.

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Compliance With Ethical Standards

Authors' Contributions

RMPG and LVL conceptualized and designed the experiment. RMPG conducted the experiment, analyzed and interpreted the data, and wrote the draft of the manuscript. LVL reviewed and edited the manuscript. Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Aypa, S. M. (1990). Mussel culture. In *UNDP/FAO (RAS/90/002), Regional Seafarming Development and Demonstration Project (RAS/90/002), Selected Papers on Mollusc Culture*. FAO. https://www.fao.org/3/ab737e/AB737E04.htm#ch4

Ballesta-Artero, I., Witbaard, R., Carroll, M. L., & van der Meer, J. (2017). Environmental factors regulating gaping activity of the bivalve *Arctica islandica* in Northern Norway. *Marine Biology, 164*(5), 116. https://doi.org/10.1007/s00227-017-3144-7

Binzer, S. B., Lundgreen, R. B. C., Berge, T., Hansen, P. J., & Vismann, B. (2018). The blue mussel *Mytilus edulis* is vulnerable to the toxic dinoflagellate *Karlodinium armiger*- Adult filtration is inhibited and several life stages killed. *PLOS ONE, 13*(6), e0199306. https://doi.org/10.1371/journal.pone.0199306

Casila, J. C., Azhikodan, G., Yokoyama, K., Fukushima, K., & Terajima, R. (2017). Effect of rainfall on saltwater and suspended sediment dynamics in multi-branched urban tidal estuaries. *JSCE Proceedings G (Environment), 73*(5), 347–352.

Cheung, S. G. (1991). Energetics of transplanted populations of the green lipped mussel *Perna viridis* (Linnaeus) (Bivalvia: Mytilacea) in Hong Kong ii: growth, condition and reproduction. *Asian Marine Biology*, *8*, 117–131.

Comeau, L. A., Babarro, J. M. F., Longa, A., & Padin, X. A. (2018). Valve-gaping behavior of raft-cultivated mussels in the Ría de Arousa, Spain. *Aquaculture Report, 9*, 68–73. https://doi.org/10.1016/j.aqrep.2017.12.005

de la Peña, M. R., & Franco, A. V. (2013). Culture of marine phytoplankton for aquaculture seed production. Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Duncan, P. F., Andalecio, M. N., Peralta, E. M., Laureta, L. V., Hidalgo, A. N., & Napata, R. (2009). Evaluation of Production Technology, Product Quality and Market Potential for the Development of Bivalve Mollusk Aquaculture in the Philippines. Final Report FR 2009-41. Canberra, Australia: Australian Center for International Agricultural Research (ACIAR). 193p.

Helm, M. M., & Bourne, N. (2004). Hatchery Culture of Bivalves: A Practical Manual. In Lovatelli, A. (Ed.), *FAO Fisheries Technical Paper 471*. Food and Agriculture Organization of the United Nations.

Ingrao, D. A., Mikkelsen, P. M., & Hicks, D. W. (2001). Another introduced marine mollusk in the Gulf of Mexico: the Indo-Pacific green mussel, *Perna viridis*, in Tampa Bay, Florida. *Journal of Shellfish Research*, *20*(1), 13–19.

Iqbal, T. H., Hajisamae, S., & Khongpuang, S. (2018). Feeding habits of Asian green mussel (*Perna viridis*): a case study from Andaman Sea and Gulf of Thailand. *Proceedings of the Asian Academic Society International Conference*, Thailand, pp. 480+484.

McFarland, K., Baker, S., Baker, P., Rybovich, M., &Volety, A. K. (2014). Temperature, salinity, and aerial exposure tolerance of the invasive mussel, *Perna viridis*, in estuarine habitats: Implications for spread and competition with native oysters, *Crassostrea virginica*. *Estuaries and Coasts, 38*(5), 1619–1628.

McFarland, K., Donaghy, L., & Volety, A. K. (2013). Effect of acute salinity changes on hemolymph osmolality and clearance rate of the non-native mussel, *Perna viridis*, and the native oyster, *Crassostrea virginica*, in Southwest Florida. *Aquatic Invasions, 8*(3), 299–310. https://doi.org/10.3391/ai.2013.8.3.06

Mero, F. F. C., Pedroso, F. L., Apines-Amar, M. J. S., Cadangin, J. F., Rendaje, D. C., Verde, C. S., Maquirang, J. R. H., & Piňosa, L. A. G. (2019). Influence of water management, photoperiod and aeration on growth, survival, and early spat settlement of the hatchery-reared green mussel, *Perna viridis*. *International Aquatic Research. 11*, 159–172. https://doi.org/10.1007/s40071-019-0226-9

Nicastro, K. R., Zardi, G. I., McQuaid, C. D., Stephens, L., Radloff, S., & Blatch, G. L. (2010). The role of gaping behaviour in habitat partitioning between coexisting intertidal mussels. *BioMed Central Ecology, 10*, 17. https://doi.org/10.1186/1472-6785-10-17

Philippines Statistics Authority (PSA). (2019). Fisheries Statistics of the Philippines 2016–2018, Volume 27. Quezon City: Philippines, Philippine Statistics Authority. 302p. https://psa.gov.ph/content/fisheries-statistics-philippines

Ponce-Palafox, J., Martinez-Palacios, C. A., & Ross, L. G. (1997). The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. *Aquaculture, 157*, 107–115. https://doi.org/10.1016/S0044-8486%2897%2900148-8

Rajagopal, S., Venugopalan, V. P., van der Velde, G., & Jenner, H. A. (2006). Greening of the coasts: a review of the *Perna viridis* success story. *Aquatic Ecology, 40*(3), 273–297. https://doi.org/10.1007/s10452-006-9032-8

Rowchai, S. (2004 abstract only). Effects of low salinity on mortality of green-lipped mussel (*Perna viridis* Linneaus). https://agris.fao.org/agris-search/search.do?recordID=TH2005001692

Segnini de Bravo, M. I. (2003). Influence of salinity on the physiological conditions in mussels, *Perna perna* and *Perna viridis* (Bivalvia: Mytilidae). *Revista de Biologia Tropical*, *51*(4), 153–158.

Segnini de Bravo, M. I., Chung, K. S., & Pérez, J. E. (1998). Salinity and temperature tolerances of the green and brown mussels, *Perna viridis* and *Perna perna* (Bivalvia: Mytilidae). *Revista de Biologica Tropical*, *46*(5), 121-125.

Soon, T. K., & Ransangan, J. (2016). Feasibility of green mussel, *Perna viridis* farming in Marudu Bay, Malaysia. *Aquaculture Reports*, *4*, 130–135. https://doi.org/10.1016/j.aqrep.2016.06.006

Su, Y., Ma, S., & Feng, C. (2010). Effects of salinity fluctuation on the growth and energy budget of juvenile *Litopenaeus vannamei* at different temperatures. *Journal of Crustacean Biology, 30*(3), 430–434. https://doi.org/10.1651/09-3269.1

Taib, A. M., Madin, J., & Ransangan, J. (2016). Density, recruitment and growth performance of Asian green mussel (*Perna viridis*) in Marudu Bay, Northeast Malaysian Borneo, three years after a massive mortality event. *Songklanakarin Journal of Science and Technology, 38*(6), 631–639.

Vu, N., & Huynh, T. (2020). Optimized live feed regime significantly improves growth performance and survival rate for early life history stages of Pangasius Catfish (*Pangasianodon hypophthalmus*). *Fishes*, *5*(3), 20. https://doi.org/10.3390/fishes5030020

Wang, Y., Hu, H., Cheung, S. G., Shin, P. K. S., Lu, W., & Li, J. (2012). Immune parameter changes of hemocytes in green-lipped mussel *Perna viridis* exposure to hypoxia and hyposalinity. *Aquaculture, 356*-357, 22-29. https://doi.org/10.1016/j.aquaculture.2012.06.001

Wang, Y., Hu, M., Wong, W. H., Cheung, S. G., & Shin, P. K. S. (2011). Combined effects of dissolved oxygen and salinity on growth and body composition of juvenile green-lipped mussel *Perna viridis*. *Journal of Shellfish Research*, *30*(3), 851-857. https://doi.org/10.2983/035.030.0326

Wu, F., Xie, Z., Lan, Y., Dupont, S., Sun, M., Cui, S., Huang, X., Huang, W., Liu, L., Hu, M., Lu, W., & Wang, Y. (2018). Short-term exposure of *Mytilus coruscus* to decreased pH and salinity change impacts immune parameters of their haemocytes. F*rontiers in Physiology, 9*, 166. https://doi.org/10.3389/fphys.2018.00166

Yuan, W. S., Walters, L. J., Brodsky, S. A., Schneider, K. R., & Hoffman, E. A. (2016). Synergistic effects of salinity and temperature on the survival of two nonnative bivalve molluscs, *Perna viridis* (Linnaeus 1758) and *Mytella charruana* (d'Orbigny 1846). *Journal of Marine Sciences*, *2016*, 9261309. https://doi.org/10.1155/2016/9261309

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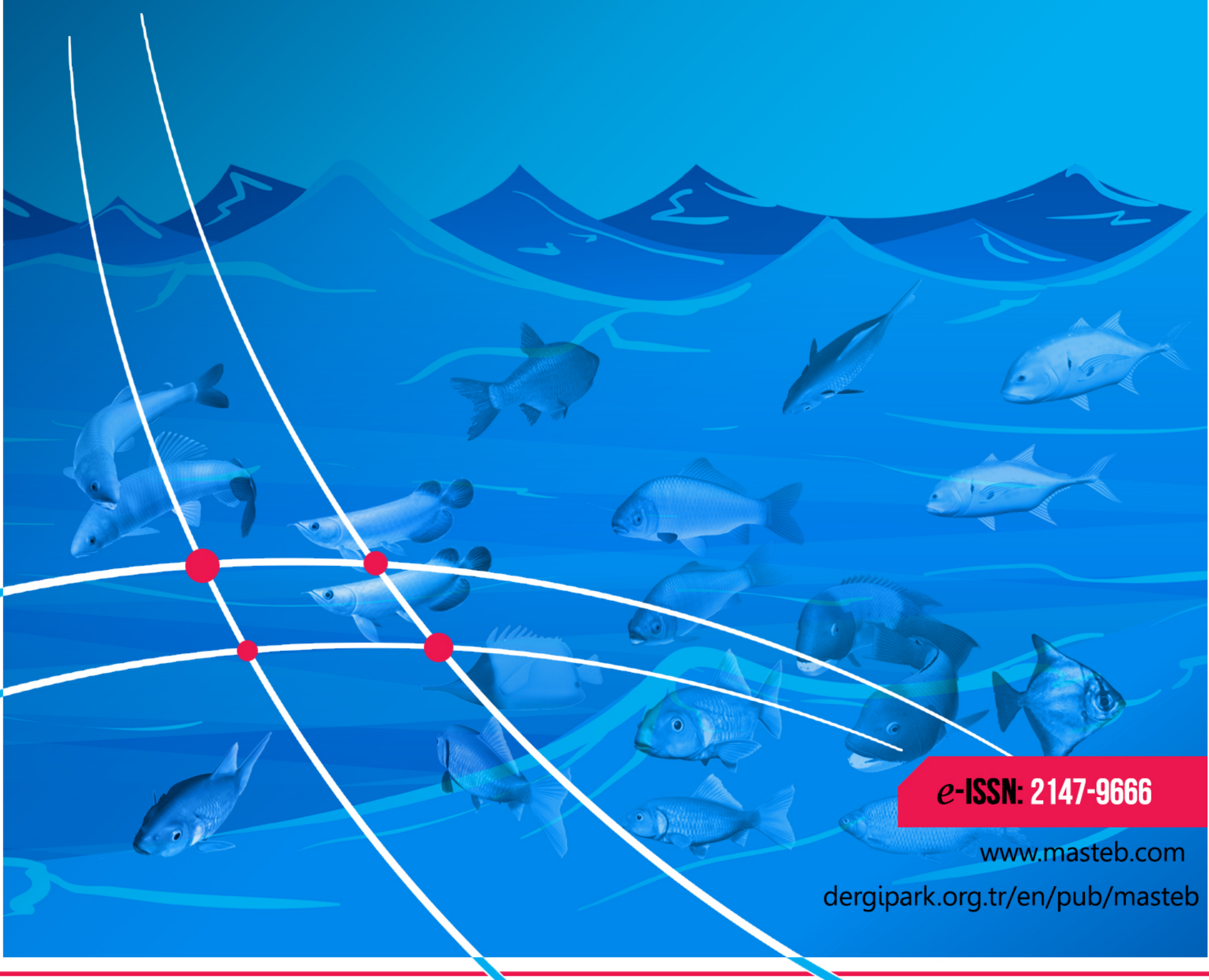

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