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Biofouling on nets and endobiosis leads to reduced shell growth in scallops (Pectinidae): A meta-analysis

Ian Cris R. Buban* and Victor S. Soliman
Coastal Resources Management Section, Bicol University Tabaco Campus
Tabaco City, Philippines
*Corresponding author: iancris.buban@bicol-u.edu.ph

Abstract

Biofouling has been generally viewed as a negative issue affecting the culture of bivalves around the world. One significant impact of biofouling is the reduction of growth of affected species. To quantify its effect on scallops, meta-analytic techniques were employed using secondary data from best available published studies. Using the random effects model, the study was able to determine a significant reduction in the growth of fouled scallop by about 24%. However, subgroup analysis revealed that the effect of biofouling on growth significantly varied depending on the type of fouling experienced by the scallops (Q=65.96, p<0.0001). Endobionts and fouling on net enclosures significantly reduced shell growth; however, growth reduction was not significant in epibiont-infested scallops. The underlying principle of the reduction in growth resulting from biofouling and its impact are discussed in this paper. The result of the study highlights the significant impact of biofouling that merits exploration of possible mitigating and control measures to address the possible impacts of biofouling for future scallop mariculture.

Keywords: *effect size, endobionts, epibionts, fouling on net, random effects model, standardized mean difference*

Introduction

Biological fouling commonly referred to as "biofouling", is the accumulation of unwanted biological matter with biofilms created by micro-organisms and macroscale biofouling (simply called macrofouling) created by macro-organisms (Bixler & Bhushan, 2012). Biofouling organisms can be classified as (a) those attached to a living surface (basibionts), which are called "epibionts" (Nys *et al*., 2010; Wahl, 1989, 2010; Wahl & Mark, 1999) and (b) those that live under the external surface of the host called "endobionts" (Sievers *et al.*, 2017; Wahl, 1989, 2010; Wahl & Mark, 1999). Most of the time, these organisms can be found attached to the nets and frames of aquaculture cages which is generally referred to as (c) "fouling on nets" (Lodeiros & Himmelman 1996).

Biofouling has been an inherent issue not just in scallop culture, but for most molluscan marine aquaculture (Adams *et al*., 2011). Different species including bivalve mollusks, barnacles, ascidians, bryozoans, sponges, and some species of polychaetes are known to form fouling communities (Carraro *et al*., 2012; Cerrano *et al.* 2006). The accumulation of these unwanted species in nets and in the scallop can affect the growth of scallop in numerous ways. One observable effect is the reduction in water flow that ultimately affects the flux of food particles available for the cultured scallops (Claereboudt *et al.*, 1994). For epibionts, it was reported to interfere with the regular vital function like the opening and closing of the shell valve (Lodeiros & Himmelman, 1996; Velez *et al.*, 1995). Considering that most of the fouling organisms are suspension feeders, there is a possible competition for food resources with the cultured scallops (Lodeiros & Himmelman, 1996; Claereboudt *et al.*, 1994). In the case of endobionts, its relationship with its host scallop has been considered as parasitism, thereby inducing stress to the latter (Baba *et al.*, 2007). Overall, biofouling in scallop aquaculture is viewed as a negative issue affecting the optimum production, although there are relatively few studies reporting lack of significant reduction on the growth of scallop resulting from such infestation (e.g., Chernoff, 1987; Lopez, Riquelme, & Gonzalez, 2000; Wallace & Reinsnes, 1985). Therefore, a systematic quantitative evaluation of best available literature on this topic would clarify whether this issue is important enough for consideration in future mariculture activities.

A quantitative review on the effect of biofouling had been previously undertaken by Sievers and colleagues (2017), but the study included multiple species of molluscs (e.g., mussel, oyster, clam, scallop, cockle, and abalone). Furthermore, the evaluations of several shellfish fitness measures (i.e., condition, flesh weight, growth, survival, size, and weight) in the previous study led to a limited number of publications on scallops (Pectinidae) that were included in the analysis. In fact, in the analysis of effect size on the growth of fouled molluscs, only one study on scallop was included. Nevertheless, it was found out that among the different bivalve fitness measured, growth was the most affected by biofouling (Sievers *et al.*, 2017).

To fully understand and quantify the reduction in scallop growth resulting from biofouling, this study was undertaken through a systematic review of best available literature making use of meta-analytic techniques for calculating effect sizes. Meta-analysis provides a rigorous framework to synthesize and compare these results through a specific set of quantitative statistical methods (Copas & Jian, 2000; Adams *et al.*, 1997; Arnqvist & Wooster, 1995). By using effect sizes, meta-analytic conclusions can be drawn by comparing standardized effect sizes across studies (Lakens, 2013), which is very useful in providing quantitative conclusion on the effect of biofouling on scallop growth. This study further quantified the effect of each type of biofouling (endobionts, epibionts, and biofouling in nets) providing more specific analysis and discussion of the underlying principles for each type of biofouling. The result will provide a basis for possible intervention which may be incorporated in future scallop mariculture.

Materials and Methods

Data selection

The scope of this study includes any research investigating the effect of biofouling on the growth of scallops. A comprehensive search of peer-reviewed literature was undertaken from September to December 2018 using Google Scholar (http://scholar.google.com). The search strategy includes the keywords "effect + of + biofouling/epibionts/endobionts + on + the + growth + of + scallops". The preliminary search returned 2,310 articles that were initially screened wherein articles not related to scallops were excluded reducing the number of articles to 154. After which, review articles, editorials, and commentaries and others that are not related to the research question were removed bringing the number of articles for further screening to 11. Finally, five studies qualified for the analysis upon checking the adequacy of the required information (Figure 1). The reference lists of the selected studies and the related studies were also examined for additional references. For this study, biofouling includes biofouling on the net enclosure, epibionts on shell surface and endobionts inside the shell, or combination of these. All the selected literature compared the growth of scallop with and without biofouling. In this case, the treatment without biofouling (clean) was designated as the control, while the treatment with biofouling was treated as the experimental group. Studies without designated control are excluded from the analysis. Only the studies that provided data (mean, standard deviation or standard error values, and sample size) are included in the analysis. Data were restricted to those studies that quantified growth rate (mm·day^{-1}) for scallops. Data on growth rate was averaged from different sampling periods across the total length of time for each study. Graphs and figures were digitized using digitizing software (PlotDigitizer; http://plotdigitizer.sourceforge.net) to generate data for the analysis.

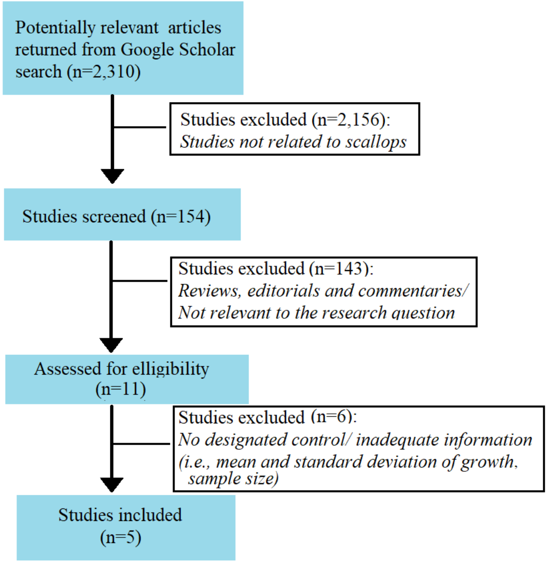

Figure 1. Flow diagram of literature search and selection process for studies included in the meta-analysis.

Some of the studies were manipulative (experimental) and some were observational. In addition, most of the studies contributed more than one data points, and in most cases have more or less than the others. For example, Claereboudt and colleagues (1994) conducted a manipulative study to determine the effect of fouling on the growth of juvenile *Placopecten magellanicus* (Gmelin, 1791) at three different depths (9, 15, and 21 m) thereby contributing three data points to the meta-analysis. On the other hand, Baba and colleagues (2007) conducted an observational study on the effect of endobionts on the growth of *Mizuhopecten yessoensis* (Jay, 1857) from samples taken in one site, thereby contributing only one data point. Combination of both manipulative and observational studies have been used to conduct a meta-analysis (e.g., Sievers *et al.*, 2017) while multiple observations within a single study were utilized due to the limited number of studies suitable for inclusion (e.g., Kerrigan & Suckling, 2016).

Data Analysis

The calculation of effect size was undertaken using standardized mean difference expressed as Cohen's *d* which was calculated using equation 1, where M^E and M^C are the mean growth rates of scallop for the experimental (with biofouling) and control group (without biofouling), respectively. According to Hedges and Olkin (1985), the use of Cohen's *d* can give a biased estimate of effect size when sample size is small (<20) or if it is different between experimental and control group. These issues were observed in this study; thus, Hedges' *g* was calculated as an unbiased corrected effect size in the meta-analysis. Values for Hedges' *g* were computed using equation 2 where n_E and n_C represent sample size for the experimental and control groups, respectively (Gurevitch *et al.*, 2001; Lakens, 2013). The variance in effect size V_D was computed as the square of standard error in effect size (SE_D) which is calculated using equation 3, where *g* is the effect size (Hedges' *g*).

$$Cohen's\ d = \frac{(M^E - M^C)}{SD_{polled}} \tag{1}$$

$$Hedges' g = Cohen's\ d\ x \left[1 - \frac{3}{4}(n_E + n_C) - 9\right] \tag{2}$$

$$SE_D = \sqrt{\left(\left[(n_E + n_C)/n_E\, n_C\right] + \left[\frac{g^2}{2} + n_E + n^C\right]\right)} \tag{3}$$

$$T = \frac{\Sigma(w_i - T_i)}{\Sigma w_i} \tag{4}$$

$$SE_T = \sqrt{\left(\frac{1}{w_i}\right)} \tag{5}$$

The random-effects model was used to compute the weighted mean effect size using the framework provided by Borenstein (2007) and Borenstein and colleagues (2010). This model was used due to the species-specific difference in growth, the difference in study site conditions (e.g., water depth, temperature, salinity, and plankton concentration), and the types of fouling (i.e., fouling on net, epibionts, endobionts) among studies. When using the random-effects model, both within study variance (V_D) and between-study variance (τ^2) are considered. In calculating between-study variance, the degrees of freedom (n-1) were subtracted from the total variance (Q) and divided by a scaling factor. For each study, the total variance was calculated by adding V_D and τ^2. In each study, weight (w_i) was computed as the inverse value of the total variance.

Multiple data points within a single study were summarized into a mean weighted effect size (T) using equation 4 (Borenstein, 2007; Borenstein *et al.*, 2010). The 95% confidence interval around the weighted mean effect size was computed from the formula provided by Borenstein (2007) and Borenstein and colleagues (2010). Finally, the summary effect size and its 95% confidence interval were calculated by combining the weighted mean effect sizes from all the studies using equations 4 and 5. The values for the effect sizes were converted into percentage to show the percent decrease in growth rate of fouled scallops. If the 95% confidence interval does not cross 0, the weighted mean effect size is considered significant (Borenstein, 2007; Borenstein *et al.*, 2010). Forest plots were generated using the method of Neyeloff and colleagues (2012) as a guide. All statistical computation and forest plots were generated using Microsoft® Excel® for Office 365 MSO (version 16.0).

Subgroup analysis of the type of biofouling

To understand how each type of fouling affects the growth of scallops, data points were grouped into three categories: (1) fouling on net, (2) epibionts, and (3) endobionts. The weighted mean effect size for each subgroup, the 95% confidence interval, and the forest plot were generated using the same method described above. Heterogeneity (variation in true effect size) was determined using Q-test, assuming the weighted mean effect sizes in each subgroup is a single study. The value of Q and p-value were computed using the formula provided by Borenstein and colleagues (2009).

Sensitivity Analysis

Sensitivity analysis was performed by testing the robustness of the result and the relative contribution of the studies particularly those with large effect sizes. Following the method employed by Kerrigan and Suckling (2016) and Kroeker and colleagues (2010), the data points were arranged from the highest to the lowest magnitude of effect sizes and the effect size with the highest magnitude (regardless of direction) was systematically removed in a stepwise fashion. The potential bias of each study was also examined by removing studies with the highest number of data points (four). After every removal of these data points and study, the meta-analysis was rerun and the effect on the weighted mean effect size was observed.

Results and Discussion

Five studies were found comparing the growth of scallop with and without biofouling. The limited studies that were included in the analysis was the result of the very specific research question focused only on scallops, specifically the effect of biofouling on its growth. Despite the limited number of studies, significant impact of biofouling on the growth of scallop was clearly observed. From the selected studies, three were manipulative, comparing growth of fouled and cleaned scallops and/or nets (i.e., Claereboudt *et al.*, 1994; Lodeiros & Himmelman, 1996; Lopez *et al.*, 2000), while the remaining two were observational studies (i.e., Baba *et al.*, 2007; and Chernoff, 1987). Three studies reported epibionts fouling, two for net fouling, and one study reported endobiont fouling. The fouling organisms and the affected scallop species are shown in Table 1.

From these studies, 13 data points were extracted and integrated into the meta-analysis. Overall, the analysis shows that biofouling significantly reduced the growth of scallops by 24.4% (Figure 2). The effect on the growth of scallops varied significantly among the types of biofouling (Q=65.96, df=2, p<0.0001). The scallops affected by endobionts have significantly lower growth than the control and those with fouling on the nets or on the shells (epibiosis). Furthermore, those scallops experiencing fouling on the nets have significantly decreased growth as compared to control, and to scallops with fouled shells (epibiosis). Interestingly, epibiont infestation in scallop shows no significant effect on growth (Figure 3).

The removal of the largest effect size and the removal of the study with four data points (i.e., Chernoff, 1987), did not alter the significance of either the weighted mean effect size across studies and the weighted mean effect

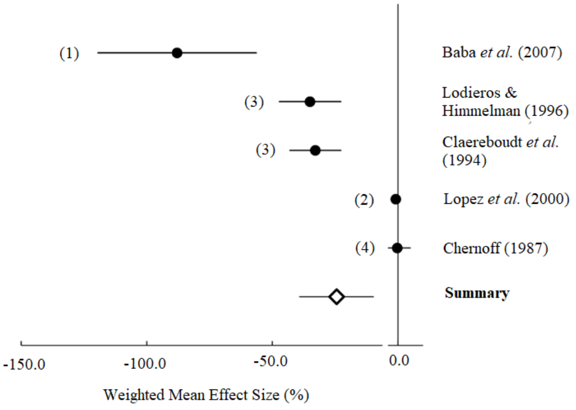

Figure 2. Forrest plot showing negative effect of biofouling on the growth of scallops (effect size and their 95% confidence interval) from different studies. The number of data points in each analysis is shown in parenthesis.

Table 1. Summary of studies showing different types of biofouling, fouling organisms and the affected scallop species.

Study	Affected species	Types of fouling	Fouling organisms
Lodeiros & Himmelman, 1996	*Euvola ziczac* (Linnaeus, 1758)	Fouling on nets and Epibionts	Bryozoans, Amphipods, Bivalves
Claereboudt *et al.*, 1994	*Pecten maximus* (Linnaeus, 1758)	Fouling on nets	Arthropoda, Mollusca, Echinodermata, Annelida, Cnidaria, Platyhelminthes
Lopez *et al.*, 2000	*Argopecten purpuratus* (Lamarck, 1819)	Epibionts	Bryozoans, Algae, Hydrozoans
Chernoff, 1987	*Chlamys asperrima* (Lamarck, 1819)	Epibionts	Sponges
Baba *et al.* 2007	*Mizuhopecten yessoensis*	Endobionts	Hydrozoan

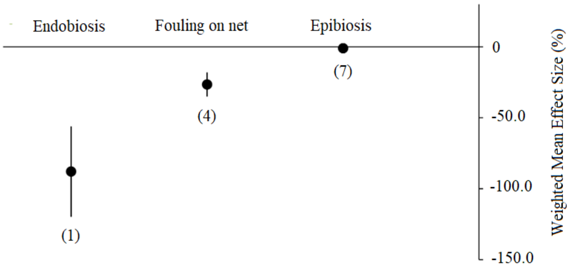

Figure 3. Forest plot showing significantly varying effects of different types of fouling on the growth of scallops (effect size and their 95% confidence interval). The number of data points in each analysis is shown in parenthesis.

size of each type of biofouling. Therefore, the result of the sensitivity analysis shows that the findings in this meta-analysis are robust.

The growth of fouled scallops was significantly lower as compared to controls underscoring the negative effect of biofouling. This has been a significant issue confronting not just the scallop growers but the entire shellfish aquaculture. In fact, a survey on the shellfish growers in the USA showed 60.9% of the respondents indicated "reduced growth" as one of the most problematic issues associated with biofouling (Adams *et al.*, 2011). The result of the present study on scallops corroborated the result of the study of Sievers and colleagues (2017) on different molluscs showing a significant reduction in growth rate. In the present study, the reduction in shell growth of scallops computed as the weighted mean effect size was 24.4%. This has significant impact in mariculture as reduced growth may be translated into smaller sizes of harvested scallops, or a longer culture period to achieve the desired harvestable size. This has led to increased production cost, specifically on labor, repair and maintenance, and energy/fuel (*Adams et al.*, 2011). Further analysis shows variations in growth in response to the different types of biofouling experienced by the scallops. Endobiosis is the type of biofouling that has the greatest impact in reducing the growth of scallops. Growth reduction resulting from endobionts infestation is significantly greater than that resulting from fouling on nets and epibionts. Fouling on nets also significantly reduced the growth of the scallops. However, biofouling by epibionts does not seem to significantly affect its growth.

Effect of Endobionts

The endobionts that live under the external surface of the host adversely affect the scallop. For example, the presence of the polyp hydroid *Eutima japonica* (Uchida, 1925) on the juvenile *M. yessoensis* decreased the scallop shell growth by 43.3%, and the relationship is best regarded as parasitism (Baba *et al.*, 2007). A study of the trophic and topical relationships of similar scallop species, *Patinopecten (=Mizuhopecten) yessoensis* and polychaete *Polydora brevipalpa* (Zachs, 1933) association showed that with abundant polychaete occupation these relationships acquire some elements of parasitism in terms of feeding activity (Silina & Zhukova, 2009). Silina and Zhukova (2009) stated reasons on why the relationship between these two species is negative: (1) feeding competition exist as evidenced by similarity of diets; (2) occupation by the polychaete results in decrease internal volume in scallop, thereby reducing the potential volume available for the scallop to filter water for respiration and feeding; and (3) the higher the polychaete occupation, the greater is the energy required for regeneration of scallops shells and the lower the efficiency of scallop growth. Similar observation from the study of Gabaev (2013) revealed that burrowing polychaete *(P. brevipalpa)* negatively affected the growth rate of *M. yessoensis* during the first and second year of cultivation.

Effect of fouling on net

Biofouling on the nets has the second most impact in significantly reducing scallop growth next to endobionts. This effect has been observed in the species *Euvola ziczac* (Lodeiros & Himmelman, 1996) and *P. magellanicus* (Claereboudt *et al.*, 1994). The result of the study of Lodeiros and Himmelman (1996) indicated that growth in shell height was more strongly affected by fouling of the pearl nets than by fouling of the shells. The growth reduction resulting from this type of biofouling can be explained by several reasons. First, the accumulation of fouling organisms reduces the water flow through the net (Claereboudt *et al.*, 1994; Lodeiros & Himmelman, 1996). From the interview in the USA, 66.4% of the mollusc growers indicated that "reduced water flow-through" was one of the most problematic issues associated with biofouling (Adams *et al.*, 2011). Fouling organisms are known to form a community in pearl nets consisting of multiple species of mollusks, arthropods, annelids, cnidarians, and platyhelminths, thus, when fouling biomass becomes high, it can block the natural flow of water (Claerboudt *et al.*, 1994). Fouling biomass on the net can be as high as 902 grams consisting of about 34 species (Claerboudt *et al.*, 1994). Furthermore, the consumption of organic particles by fouling organisms (mostly filter feeders) may compete and limit the availability of food for the scallops (Claereboudt *et al.*, 1994; Cote *et al.*, 1994; Lodeiros & Himmelman, 1996). As an evidence, an experiment conducted on the scallop *Chlamys nobilis* (Reeve, 1852) found out that the chlorophyll-a uptake rate and the consumption of particulate matter significantly increase in fouled scallop as compared to cleaned scallop (Zhenxia *et al.*, 2008). The more diverse the community of fouling organisms, the more efficient is the consumption of food resource (Mook, 1981). According to Mook (1981), fouling community modify the seston size since they filter particles in a range from 1 to 40 µm, and release sizes from 1.5 to 5.0 µm as pseudofeces. These smaller particles are received by scallops, but their nutritional value is too low to give them the necessary energy to grow (Claereboudt *et al.*, 1994). Cahalan and colleagues (1989) conducted an experiment subjecting the juvenile *Argopecten irradians* (Lamarck, 1819) at varying flow velocity and food concentration. The result revealed that the effect of food concentration is much more pronounced than the effect of flow velocity. Thus, it can be summarized that the negative effect of biofouling in the net can be influenced by competition in food source between the scallops and the biofouling organisms, and the effect may be more pronounced in areas and seasons with low food concentrations.

Effect of epibionts

Epibionts also lowers growth of scallop, but only for as little as 1% which is not significant. Although it has been previously assumed that their presence may interfere with the normal vital function of the host scallop such as the opening and closing of the valve (Lodeiros & Himmelman, 1996; Velez *et al.*, 1995), the result of the meta-analysis shows that this effect may not be translated into significant growth reduction. However, it is important to note that most of the data in this subgroup came from purely observational study in which according to Sievers and colleagues (2017), initial difference between fouled and unfouled scallop may exist. Furthermore, these observational data were results of fouling of a single species alone (sponge) (see Chernoff, 1987) instead of the usual epibiont community consisting of multiple biofouling species. This has significant bearing to the result of the meta-analysis, since some species of sponge were observed to have a positive relationship with scallops (Armstrong *et al.*, 1999; Chernoff, 1987; Pond, 1992). Furthermore, low biomass of epibionts infestation was also reported leading to insignificant growth reduction. An experimental study conducted by Lopez and colleagues (2000) on the fouling of the scallop *Argopecten purpuratus* assumes that the low biomass of epibionts cause lower competition for food, hence no significant reduction in growth was observed. Therefore, the non-significant reduction in growth resulting from epibiont infestation observed in this study may not necessarily translate into a complete lack of effect.

The necessity for biofouling mitigation and control

The effects of the different types of fouling have been described separately in this study. However, in the natural setting, it is most certain that these types of fouling will occur simultaneously. In the study of Lodeiros and Himmelman (1996), the greatest decrease in scallop growth was observed in the combination of fouling on net and epibionts. Therefore, possible measures must be considered collectively to address these different types of biofouling concerns. One important consideration is understanding the natural recruitment of biofouling organisms to avoid their settlements in aquaculture facility and cultured species (Fitridge *et al.*, 2012). Several control measures have been used to control biofouling in scallops which include the consideration of culture depth (Berkman, 1994; Claereboudt *et al.*, 1994), location (Lopez *et al.*, 2000) and a range of chemical (Davies *et al.*, 1986; Davies & Paul, 1986; Paul & Davies, 1986; Tettelbach *et al.*, 2014) and biological control (Dumont *et al.*, 2009; Ross *et al.*, 2004; Zhenxia *et al.*, 2008). Biological control, i.e., the use of various animals to control the settlement and growth of biofouling species, is a comparatively more effective anti-fouling strategy in terms of its impact on stock fitness, since some of the strategies (e.g., manual, mechanical and chemical removal) have its drawbacks (Sievers *et al.*, 2017). Thus, further study should be conducted to explore the effects of the reported biological control measures on biofouling on the cultured scallops.

Conclusion

The result of the present study provides a clear understanding of the impact of biofouling on scallop growth. Biofouling, particularly endobiotic infestation and fouling on nets, leads to significant reduction in the growth of scallops where slower growth can be translated into longer culture period or smaller size at harvest. Thus, this should be viewed as a serious concern for scallop culture. Considering how endobionts and biofouling on nets significantly lower shell growth, efforts should be focused on how to minimize their impact. This should include understanding the biofouling community and their seasonality of occurrence in an area, and exploration of the possible use of biological control organisms. The result of this study will guide the future mariculture operations.

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