

## Research Article

# Cultivated brown mussel (*Perna perna*) size is reduced through the impact of three invasive fouling species in southern Brazil

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## Abstract

Invasive species reduce the productivity of shellfish mariculture worldwide. Brown mussel culture harvests were examined for invasive species in the state of Santa Catarina – the most important region for shellfish mariculture in Brazil. For the first time, we describe here the impact of the three most abundant invasive species on harvested *Perna perna*. The ascidian *Didemnum perlucidum*, the barnacle *Megabalanus coccopoma*, and the bryozoan *Schizoporella errata* were all associated with smaller mussel size. Fouled mussels were 19–36% smaller and weighed ~ 60% less than non-fouled mussels. Reductions in mussel size were greatest for shell weight and size when associated with *D. perlucidum* and tissue dry weight for *M. coccopoma*. This large reduction in productivity indicates that management of these fouling species should be prioritized to protect the mussel fishery.

**Key words:** *Didemnum perlucidum*, *Megabalanus coccopoma*, *Schizoporella errata*, condition index, shellfish mariculture

## Introduction

Mariculture often faces financial challenges due to fouling species because they reduce productivity and add costs associated with cleaning gear and maintaining equipment. A large number of fouling species are introduced outside their native distribution and many are also invasive (Rocha et al. 2009; Fitridge et al. 2012). Species become invasive if the barriers that previously limited population growth are removed, with individuals widespread at multiple sites across the extent of occurrence (Blackburn et al. 2011). In many regions, several invasive fouling species may be present. Risk assessment, therefore, must examine the regional fouling community to determine the importance of each species to guide targeted management plans where invasive species are common or likely to become a problem (Fitridge et al. 2012).

An example of an exotic fouling species (i.e. epibiont) with devastating impacts on mariculture is the ascidian *Ciona intestinalis* in Canada, which can become extremely abundant and increase mortality of a native mussel

*Mytilus edulis* (Daigle and Herbinger 2009), thereby reducing crop size and increasing production costs. Costs due to fouling vary geographically because they are a consequence of the composition of the epibiont community, abundance and recruitment regimes of the species involved and their temporal dynamics, all of which vary regionally (Lacoste and Gaertner-Mazouni 2015). To date, estimates on the impacts of fouling species, often including ascidians, suggest that up to 30% of production costs go towards combating fouling organisms (Watson et al. 2009; Dürr and Watson 2010; Adams et al. 2011; Fletcher et al. 2013).

The brown mussel *Perna perna* is regulated in Brazil as a native commercial species (*Instrução normativa IBAMA No. 105, 20/7/2006*). For more than 20 years, the southern state of Santa Catarina has led Brazilian brown mussel production. The market of over 20 thousand tons of mollusks provides a substantial source of revenue for the ~ 550 independent mussel producers (Santos and Della-Giustina 2017). Most producers are family-owned artisanal businesses, and impacts due to fouling species may challenge the sustainability and growth of this important commodity. One of the most productive regions in the state is in the Itajaí river basin, the largest watershed flowing into the Atlantic Ocean. Here, in the city of Penha, productivity is negatively affected by fouling species that include many exotic ascidians (Rocha et al. 2009). This is also likely to be an important recipient region for the introduction and spread of additional species due to its proximity to the international port of Itajaí, one of the largest ports in Brazil (ANTAQ 2018).

Three species, the colonial ascidian *Didemnum perlucidum* Monniot, 1983, the barnacle *Megabalanus coccopoma* (Darwin, 1854), and the cheilostome bryozoan *Schizoporella errata* (Walters, 1878), are among the most common fouling species in suspended mussel farms in Penha (*personal observation*). They are also common in many ports, harbors, marinas and on associated artificial structures (Hedge and Johnston 2012); transport by shipping is considered the most common pathway to introduction and spread of these species. The ascidian *D. perlucidum* is widespread globally, and locally it has been introduced in Santa Catarina mussel farms for at least ten years (Kremer et al. 2010; Dias et al. 2016). *Didemnum* colonies overgrow mussels and can completely envelope the valves. Colonies can also grow over barnacles and other sessile organisms (Culbertson and Harper 2000), even though it might be a weak competitor for primary substrate (Kremer and Rocha 2011). The barnacle *M. coccopoma* recruits rapidly on disturbed as well as primary substrates (Newman and McConaughey 1987). Because the barnacle has sharp edges, infestation can degrade ropes and injure farmers if not handled with great care. Removal of barnacles by scraping from harvested mussels can be extremely time-consuming. The barnacle was first reported in Brazil in the 1970s (Lacombe and Monteiro 1974; Young 1994) and is the only fouling species

on the Santa Catarina exotic invasive species list (FATMA 2016). The bryozoan *Schizoporella errata* is a widespread, subtropical, shallow water fouling species that grows vigorously on sea-farm structures (McKinney and McKinney 2002). In Penha, it fouls mussel shells, ropes and buoys with heavily calcified purple/brown colonies (called “rust” by the fishermen) that are up to 20 cm in diameter when growing on buoys (*personal observation*). Although listed in the official Brazilian list of invasive exotic species (Lopes 2009), recently *S. errata* has been conservatively considered cryptogenic because of taxonomic issues in which the name comprises a complex of species (Miranda et al. 2018).

To better understand the impact of these fouling species on mussel culture, we tested how each of the three focal taxa affects mussel yield and predicted that fouled mussels would exhibit reduced size and/or weight when compared to unfouled mussels in traditional artisanal shellfish cultivation in Brazil.

## Materials and methods

### *Mussel farming*

Mussels were sampled at mussel farms in the Armação do Itapocoroy bay in Penha, Santa Catarina (26°46'30"S; 48°36'34"W). Here, mussel farming begins with brown mussel “seed” that is collected from natural banks on rocky shores. Mussel seed is graded using a 3 cm grid and then placed in production. A 2 m rope with filamentous loops for mussel attachment is inserted into a 2 m long tubular cotton cloth bag (the cultivation sock). About 800 mussel seeds are poured into each sock and then placed in the cultivation grid. Harvest occurs eight to ten months after socking and, when harvested, socks weigh ~ 40 kg. Mussel seed density does not affect production (i.e. shell size and flesh weight are constant over normal densities in the socks, Suplicy 2018). Our sampling was carried out as the mussels were being harvested and thus are representative of typical sizes at harvest time.

### *Sampling methods*

We sampled mussels during the austral summer months of November–February of 2016–2018, when surface water temperature varied from 23–27 °C and salinity from 32 to 35 PSU. We first examined several mussel farms in Santa Catarina to determine which fouling species were important and amenable to study. We examined mussel socks to find individual mussels clear of fouling invertebrates and algae and with no evidence of prior encrustation (hereafter referred to as the control) as well as mussels fouled by at least one of the focal invasive species (at least one valve covered > 70%): *Didemnum perlucidum*, *Megabalanus coccopoma*, or *Schizoporella errata* (hereafter referred to as “fouled”). We rarely found all three target

species in the same sock; therefore, we collected all the fouled mussels covered by only one of the targets and respective controls in each sock. Controls were selected from the same sock as the fouled individuals and so all samples by sock contain fouled and control individuals of the same age. However, because fouling is intense, the minimum number of controls of two was often less than the number of fouled individuals. If we were unable to find controls in a sock, we did not use that sock in any analysis. Occasionally, small individuals were found in socks, but they clearly do not belong to the same cohort as that of the majority of mussels in each sock, and those individuals were not included in analyses. Each sampled mussel was individually placed in a sealed bag, labeled and frozen prior to processing.

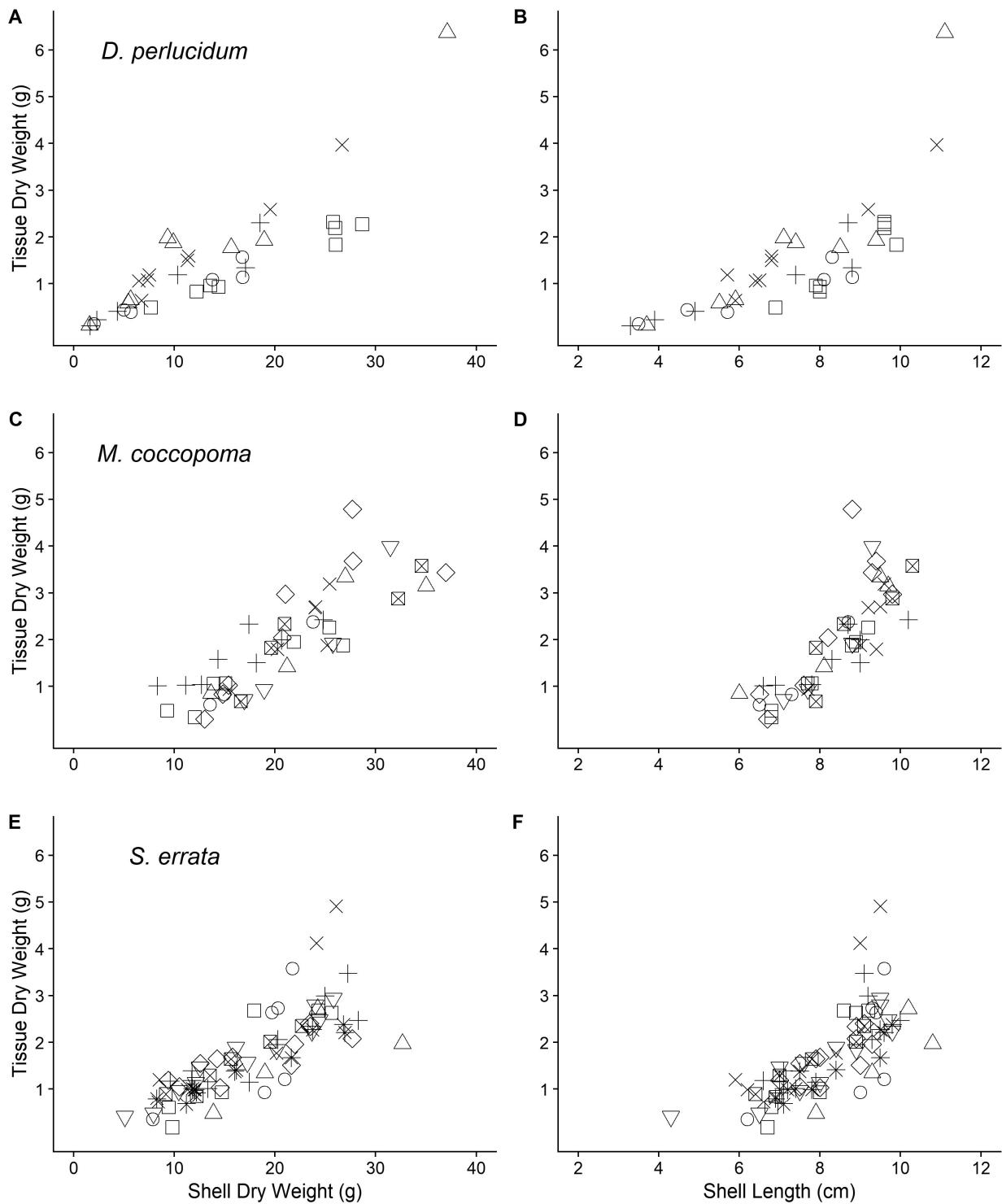
In the laboratory, shell length was measured to the nearest 0.5 mm using a digital caliper. Besides shell length, the weight of mussels is also important to the market and so, fouled mussel-shells were scraped clean, heated for 5 min at 91–96 °C and dissected. Then, shell and tissue of each sample were desiccated for 48 h at 60 °C and then weighed to 0.001 g precision on a digital balance. A condition index that indicates the tissue percentage of total individual dry weight was calculated as  $\text{dry weight}_{\text{tissue}} / (\text{dry weight}_{\text{tissue}} + \text{dry weight}_{\text{shell}}) \times 100$  and is the percentage of total mussel weight due to tissue (Davenport and Chen 1987).

### Statistical analysis

The effects of fouling on mussel yield were tested using one-sided t-tests predicting that mussel size was reduced by fouling. The following four variables of mussel size were tested: shell length, dry shell weight, dry tissue weight and condition index. Because all foul-control samples of socks comprised one fouling species in each sock, we analyzed each fouling species separately. Our sampling did not permit testing for interactions due to fouling by more than one species. If an effect due to fouling was established, we compared the effect size of each fouling species as the difference in the variables of the fouled individuals subtracted from the mean values of those variables from their respective controls, using analysis of variance. Because all socks were measured at the time they were harvested, we assumed that socks were independent of the effect of fouling, but examined the potential of that effect in scatterplots. All tests were considered significant at  $\alpha = 0.05$ . Statistical analyses were carried out in R 3.5.3 (R Core Team 2019).

## Results

Most mussels in each sock had several fouling species and few had none at all. Thus, while difficult to find mussels in each sock fulfilling our criteria, we collected 21 mussels fouled by *D. perlucidum* with 15 controls in five socks,



**Figure 1.** Scatterplots of mussel dry tissue weight as a function of two size measurements (dry shell weight, shell length) of fouled samples to illustrate that there is no apparent effect of sock of origin (symbols only indicate different socks, so symbols are independent of species – that is, the same symbol for a different species indicates a different sock). A) and B) – *Didemnum perlucidum*, n = 5 socks, C) and D) – *Megabalanus coccopoma*, n = 8 socks, E) and F) – *Schizoporella errata*, n = 9 socks.

24 mussels fouled by *M. coccopoma* with 22 controls in eight socks, and 41 mussels fouled by *S. errata* and 31 controls in nine socks.

Scatter plots of mussel measurements demonstrated that socks did not influence mussel size, as we assumed, given that mussels from different socks were not grouped together (Figure 1). Thus, we did not include sock

**Table 1.** Summary of *t*-tests comparing size and quality measurements of harvested brown mussels (*Perna perna*) between control and fouled treatments. All *t*-tests were statistically significant, with Control > Fouled, at P < 0.001, except Condition index, in *D. perlucidum*, where P = 0.627.

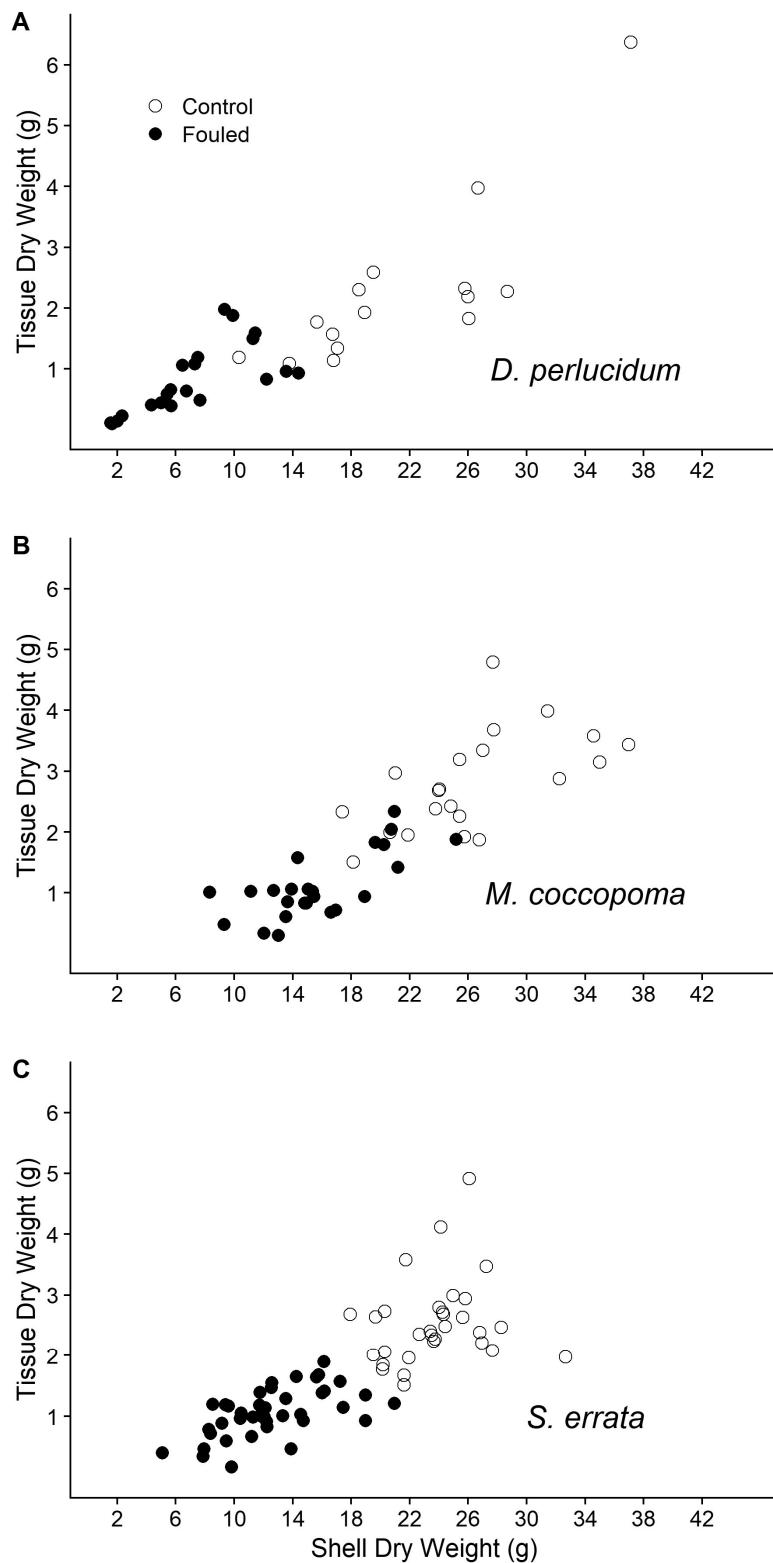
Variable	Control	Fouled	<i>t</i>
<i>Didemnum perlucidum</i> (df = 34)			
Length	9.19	5.93	7.42
Dry tissue weight	2.25	0.82	4.39
Condition index <sup>1</sup>	9.31	9.66	0.33
<i>Megabalanus coccopoma</i> (df = 44)			
Length	9.32	7.54	8.76
Dry tissue weight	3.00	1.11	6.95
Condition index	10.10	6.44	4.80
<i>Schizoporella errata</i> (df = 70)			
Length	9.34	7.33	10.82
Dry tissue weight	2.54	1.07	11.24
Condition index	9.71	7.84	3.42

<sup>1</sup> Condition index = dry weight<sub>tissue</sub>/(dry weight<sub>tissue</sub> + dry weight<sub>shell</sub>) × 100.

as a covariate in any of the subsequent analyses. Fouled mussels tended to be smaller, often substantially so, in all measurements and for all fouling species (with the exception of the condition index in *D. perlucidum*, Table 1, Figure 2). Fouled mussels weighed less than half as much as control mussels (*S. errata*, 42%) to just more than one third as much (*D. perlucidum*, 36%, and *M. coccopoma*, 37%). There was no evidence for allometric influences during growth or fouling of any of the target species, nor did sock influence any of the measurements (Figure 2). Thus it was clear that fouled individuals simply tended to be smaller. The effect size (the reduction in growth due to fouling) was greatest in *D. perlucidum* on shell weight and length, and on tissue dry weight for *M. coccopoma* (Table 2, Figure 3).

## Discussion

Fouling by the ascidian *D. perlucidum*, the barnacle *M. coccopoma* and the bryozoan *S. errata* all clearly impacted the size of the brown mussel. These mussels were all collected from socks at the time of harvesting; if they had not yet reached general commercial standards (length > 9 cm) at this point in time, it was likely due to fouling. Fouled mussels were as much as 36% smaller and weighed ~ 60% less than expected at harvest. Consequently, it was clear that not only will fouled mussels take longer to reach marketable size, but many may never reach marketable standards. Once harvestable size has been reached in a sock, the harvesting practice is to simply collect all individuals because after that time, growth rate slows, mortality and fouling increases, and parasitism may also increase (Baird 1966; Holt et al. 1998). Thus, simply leaving the sock out for longer to allow fouled mussels to increase in yield will not compensate for the losses. Controlled experimental studies have also demonstrated that mussel yield declines after variable periods of growth and circumstances due to fouling. Managed ten-month-old mussel socks that were cleaned monthly produced

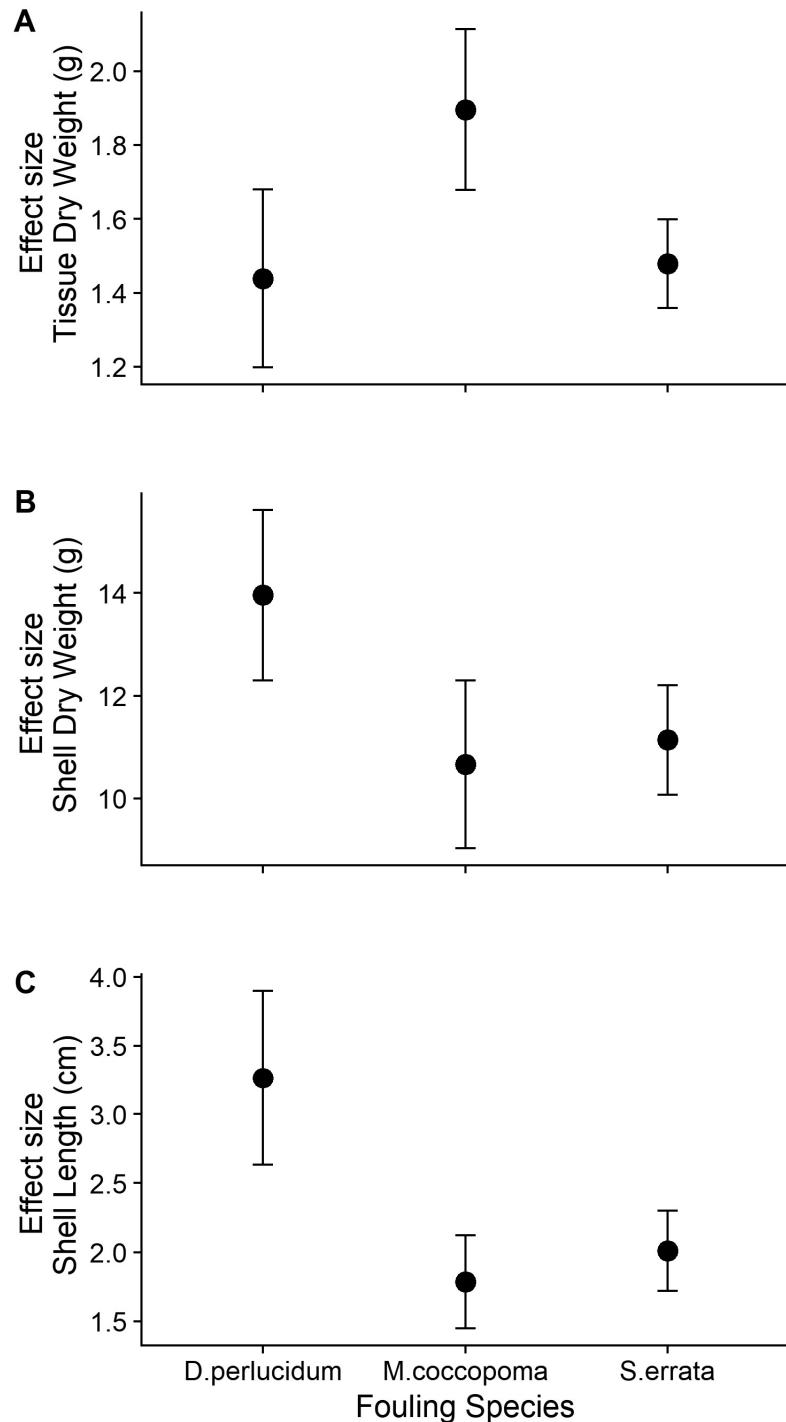


**Figure 2.** The impact due to fouling was mainly due to reduced growth as shown in scatter plots of tissue dry weight by shell dry weight, by fouling species. A) *Didemnum perlucidum*, B) *Megabalanus coccopoma*, C) *Schizoporella errata*.

brown mussels that were 7% longer and 15% heavier in flesh weight than socks that were not cleaned (Sá et al. 2007). The mussel *Mytilus galloprovincialis* fouled by the ascidian *Ciona robusta* for only two months

**Table 2.** Comparison of the effect size (control minus fouled) among fouling species on three variables of size and quality in the brown mussel (*Perna perna*). Underlined values indicate those that are statistically different from the others (Tukey's post hoc test). See Figure 3.

Variable	<i>Didemnum perlucidum</i> (N = 21)	<i>Megabalanus coccopoma</i> (N = 24)	<i>Schizoporella errata</i> (N = 41)	F	P
Tissue Weight (g)	1.43 (0.56)	<u>1.89 (0.54)</u>	1.47 (0.39)	6.93	0.001
Shell Weight (g)	<u>13.90 (3.87)</u>	10.60 (4.08)	11.1 (3.48)	5.17	0.007
Length (cm)	<u>3.26 (1.48)</u>	1.78 (0.84)	2.00 (0.94)	12.6	<0.001



**Figure 3.** Comparison of effect size (mean control value minus the individual fouled sample values) among fouling species in three size and quality variables in the brown mussel (*Perna perna*). A) Tissue dry weight (g), B) Shell weight (g) and C) Shell length (cm). Statistical results in Table 2.

were shorter (by 4%) and smaller (flesh weight reduction of 21%) than unfouled mussels (Sievers et al. 2013). *Mytilus galloprovincialis* fouled by the hydroid *Eudendrium crocea* were also shorter (4%) and lighter (23%) after six months (Fitridge and Keough 2013). Our data also demonstrated a substantial decline in size and weight of fouled mussels.

Mussels can respond to stress and, when faced with environmental challenges such as fouling, can invest less energy in growth and reproduction (Petes et al. 2008). Surprisingly, the condition index of mussels fouled by *D. perlucidum* was similar to that of controls, yet fouled mussels were much smaller (Table 1). This indicates a problem with using the condition index as a measure of mussel quality and the impact from fouling. The condition index is widely used as a tool to determine if mussels are at their best cost-benefit relationship for consumers (Okumuş and Stirling 1998; Peharda et al. 2007). However, to be effective, this index requires that mussels be of the same size or that the index is used to compare those growing at the same allometric rates. If those conditions are not met, small shelled mussels may have relatively large flesh and so the condition index will be a large number, while large shelled mussels may have proportionally smaller flesh and hence a lower index value. However, the large, low-index value mussel can have a much larger flesh than the small, large-index value mussel. Because the goal is to cultivate larger quantities of marketable flesh, using the condition index during mussel cultivation can result in misleading conclusions when small mussels have large condition values.

Mussels seem to be impacted by fouling species because they compete for food (Woods et al. 2012; Sievers et al. 2013) or grow over the valves such that they hinder typical water flow in some way, perhaps by impeding shell opening (Lodeiros and Himmelman 1996, 2000). The impact of *D. perlucidum* was greater than that of the other two species on shell weight and size. This is surprising because *D. perlucidum* is less calcified and more flexible than the other fouling species, which suggests that it should interfere less with valve growth. For instance, size and condition were not reduced in the green-lipped mussel *P. canaliculus* fouled by another invasive co-generic colonial ascidian, *Didemnum vexillum*, after 15 months of exposure (Fletcher et al. 2013). *Megabalanus coccopoma* caused a greater reduction in tissue dry weight, suggesting that its greater weight might interfere with shell movement and either decrease feeding or increase energy use by mussels when opening and closing heavier shells (Lodeiros and Himmelman 1996, 2000). The mechanisms causing detrimental consequences of mussel fouling should be further studied to better understand what possible control mechanisms, and thus management options, might be feasible. For instance, competition for food suggests that regular cleaning of infrastructures should be carried out along with cleaning the mussels themselves.

Several methods are used to clean fouling species during production, including exposure to air, immersion in a variety of water-based treatments,

and pressure washing. All of these may influence mussel growth as well (Sievers et al. 2017), and none has been adequately tested to determine the cost-benefit relationship with the three fouling species examined in this study. Management of fouling species is time consuming and expensive, especially for artisanal farmers that do not have the machinery and employees required. Handling costs (in time, effort and money) may also differ depending upon the fouling species. For example, *D. perlucidum* and *S. errata* can be removed with very small risk of damaging mussels, while the barnacle *M. coccopoma* must be manually scraped from each mussel. Also, by-products of cleaning fouling animals themselves must be disposed of appropriately. For instance, colonial tunicates, if returned to the water, are likely to invade additional locations or simply reinfect the farm where they were cleaned (Paetzold and Davidson 2010). Hard shells of some animals, such as barnacles, may then become substrate for attachment of exotic species if shells accumulate on the soft bottom beneath the cultures. Therefore, until studies specifically address the costs, benefits and environmental consequences of cleaning, it is suggested that farmers do not attempt to remove fouling species from shells during mussel growth (Metri et al. 2002; Lodeiros et al. 2007) and limit efforts to cleaning the finished product. For example, only clean when the external shell appearance matters, such as in local markets, as opposed to commercially shelled and cooked mussels.

Rather than *in situ* cleaning, a better management plan would be to adjust timing of harvesting to avoid the time during which fouling is at its peak. Reproduction by *D. perlucidum* and *M. coccopoma* may occur throughout the year at Penha city, but *M. coccopoma* tends to appear in early summer and reach its greatest settlement during the summer (Severino and Resgalla 2005), while *D. perlucidum* reaches its greatest abundance (biomass) in the summer and settlement rates are greatest in March (Kremer et al. 2010), after mussels are harvested. The bryozoan *S. errata*, similarly, reproduces most during warmer months in another subtropical region (Sutherland and Karlson 1977). Given that most farmers prepare (seed) mussel socks within a brief time interval (weeks to a couple months) starting in March, harvesting of eight to nine-month-old mussels between November and December would avoid the time of greatest abundance of these fouling species. Following this recommendation would result in mussels that could be marketed as shelled and cooked to be preserved for export. In the Armação do Itapocoroy bay, optimum brown mussel commercial standards are usually reached at about seven months, with a maximum increase of > 3.5 g in flesh weight from October to November (Marenzi and Branco 2005). However, mussels are harvested throughout the summer (December–February) because it is the most important tourist season, and larger shelled mussels tend to be preferred when bought fresh on site, which is how they are typically served in Brazilian restaurants.

Here, we clearly show that three epizootic species that are dominant in mariculture fouling can cause a substantial reduction in mussel productivity. The list of Brazilian invasive exotic species does not mention *D. perlucidum* and defines both *M. coccopoma* and *S. errata* only as established (Lopes 2009), and thus should be updated. The status of *S. errata* is still in dispute, and in a more conservative view is recently considered cryptogenic because of the lack of information about its geographical origin (Miranda et al. 2018). On the other hand, being exotic is moot because, if we use the definition of Valéry et al. (2009), the species is indeed invasive to the cultures, and its impact on mussels suggests the necessity of management. Because of similar environmental conditions, the ~ 100 ha of the Armação do Itapocoroy bay mussel farms is probably already invaded by all three fouling species. These three invasive species are also found in other mariculture operations in the state of Santa Catarina (*unpublished data*). To reduce the likelihood of transport of non-native species, stakeholders must be aware that invasive species can “hitch-hike” between regions that are producing bivalves. Current Brazilian law requires that a document must accompany mussel shipments and describe the animals being transported (*Guia de Transporte Animal – GTA*). However, this is insufficient because the document does not require information of the epizootic invasive species that may be present nor is the document checked by biologists during transport or at their destinations.

In conclusion, we emphasize the need for further study to examine the cost-effectiveness of fouling mitigation in Brazilian mariculture. Based on their impact, these three species should be considered as priorities for any management action. Recruitment of fouling species should be continually monitored so that cleaning socks and infrastructures can be undertaken at times that would be most effective. Harvesting may be carried out before invasive species reach high densities. Regional coordinated strategies to mitigate fouling species impacts should be implemented and are highly recommended to prevent the reinfection of structures and the transport of associated species between regions.

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