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Research Article

Mitigating non-indigenous species movements: effects of pressure-washing intensity and duration on the removal of biofouling and mobile invertebrates from cultured Pacific oysters (*Crassostrea gigas* (Thunberg, 1793))

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Abstract

The inadvertent movement of non-indigenous species (NIS) poses a significant risk to marine ecosystems. The present study examined the interactive effects of pressure-washing intensity and duration on removal of biofouling and various mobile invertebrate species on string-cultured Pacific oysters (*Crassostrea gigas* (Thunberg, 1793)). Six pressure-washing treatments were established by combining two intensities (2000 and 3000 PSI) and three durations (10, 20, and 30 s). These were compared with controls of no washing and simple dunking, which most likely are the current industry practices. Oysters in the various pressure-washing treatments had significantly less total biofouling compared to the no-washing and dunk controls. Significantly less biofouling remained when pressure washing was applied for longer periods of time (20 and 30 s) than for shorter periods (10 s), regardless of intensity. Dunking oysters repeatedly in seawater had no significant effect on the amount of biofouling when compared with the no-wash control, although it did lead to significantly fewer shrimp. Regardless of the faunal group assessed (i.e. total biofouling community, NIS tunicates, or various mobile invertebrate species), individuals remained on the oysters after every experimental washing treatment, suggesting none are 100% effective. In addition, the number of oysters remaining on the strings and their shell condition were significantly reduced after pressure washing, suggesting a potential cost to growers. The results have implications both for oyster farming and mitigation of NIS movement.

Key words: crabs, invasive tunicates, non-indigenous species management, pressure washing, shellfish aquaculture

Introduction

Movements of wild-caught or cultured shellfish have been identified as a vector for the introduction and spread of many non-indigenous species (NIS) around the world (Minchin 2007; Curtis et al. 2015; Grosholz et al. 2015). Ruiz et al. (2000) estimated that both fisheries (including aquaculture) and shipping were responsible for 76% of secondary NIS introductions in North America, with those being attributed directly to fisheries (15%) being dominated by NIS translocations associated with oyster movements.

In addition, Grosholz et al. (2015) estimated that 106 NIS introductions associated with aquaculture have occurred over the years in California, USA alone. These NIS encompassed 13 different taxa, but were predominantly crustaceans and molluscs (~ 50% of hitchhiking NIS) (Grosholz et al. 2015). Looking only at the Strait of Georgia, Salish Sea, British Columbia (BC), Canada, Levings et al. (2002) also showed multiple hitchhikers associated with Pacific and Atlantic oyster imports.

On Vancouver Island (VI), BC there are four marine NIS of particular concern – the European green crab (*Carcinus maenas* (Linneaus, 1758)) and three colonial tunicates (golden-star tunicate (*Botryllus schlosseri* (Pallas, 1776)), violet tunicate (*Botrylloides violaceus* Oka, 1927), and carpet sea squirt (*Didemnum vexillum* Kott, 2002)). These have all been found on cultured Pacific oysters (*Crassostrea gigas*) and associated with Manila clams (*Venerupis philippinarum* A. Adams and Reeves, 1850) harvested from the west coast of VI, destined for processing plants, which are only located on the east coast of the island and lower mainland (Curtis et al. 2015). The three tunicate species are found in various locations all around the BC coastline, but there are many areas potentially devoid of them (Gartner et al. 2016; DFO 2018), thus there are concerns about furthering their spread via various vectors, including cultured shellfish movement. Green crabs have well established populations on the west and south coasts of VI and there is a particular concern that the species could be transported from existing populations to the Strait of Georgia (on the east side of VI) through cultured shellfish movements to processing plants (Curtis et al. 2015; Ferguson et al. 2017). Green crab is extremely tolerant to a wide range of salinity and temperature regimes (Therriault et al. 2008), a voracious predator of shellfish and other marine invertebrates (Grosholz et al. 2000, 2011), and can destroy eelgrass beds (Matheson et al. 2016; Howard et al. 2019), all of which contribute to it being a known high risk global invader. As a result, federal conditions of licence for BC shellfish growers on the west coast of VI stipulate that they must rinse their product after harvest, prior to transport to processing facilities (DFO 2016), but there is no elaboration on what rinsing should entail.

Due to the concern of spreading NIS on transferred shellfish, there has been some research examining potential mitigation measures, including various physical (brushing, manual picking, pressure washing, scraping, scrubbing), biological (sea urchins, shrimps, whelks), and chemical (acetic acid, bleach, brine, lime) treatments (see Forrest et al. 2007; Epelbaum et al. 2009; Piola et al. 2009; Paetzold and Davidson 2010; Switzer et al. 2011; LaCoste et al. 2014; Sievers et al. 2017). However, most have focussed on the removal of invasive colonial tunicates (i.e. Arens et al. 2011; Paetzold et al. 2012), with relatively little research examining mobile species such as crustaceans, echinoderms, and gastropods (but see Pit and Southgate 2003; de Sá et al. 2007; Marenghi and Ozbay 2010; Calderwood et al. 2016).

Physical removal of biofouling NIS, like other control/mitigation techniques, varies in its efficacy and practicality (Fitridge et al. 2012). For instance, scrubbing has been used to assess the impacts of biofouling on shellfish growth (e.g. growth increment, tissue mass, biomass) (Claereboudt et al. 1994; Lodeiros and Himmelman 1996; Pit and Southgate 2003; de Sá et al. 2007), but few studies have quantified the change in biofouling due to manual removal. The exception is Switzer et al. (2011), in which it was found that scrubbing removed 30 to 70% of the biofouling coverage on Pacific oysters when compared to the control. Impacts on shellfish vary with some finding lower survival and total wet weight of the cultured species (Pit and Southgate 2003; Switzer at al. 2011). While picking, scrubbing, and pressure washing can remove some of the biofouling they may stress and damage cultured shellfish, are extremely labour intensive, and likely not cost-effective methods for industry to apply (Switzer et al. 2011; LaCoste et al. 2014; Sievers et al. 2017).

Although pressure washing shellfish to remove NIS may be less time consuming and costly than manual picking and hand scrubbing, few studies have examined its efficacy. Arens et al. (2011) reported that 40 and 67% of the biofouling community (target species *B. schlosseri*) was removed by pressure washing at 40 and 700 PSI, respectively, for 10 s. Likewise, Paetzold et al. (2012) found that 67% of the total epifaunal community was removed when pressure washed at 700 PSI for 10 s. The greatest efficacy was reported by Forrest and Blakemore (2006) who found that 90% of *Undaria* gametophytes were removed by pressure washing at 1000 PSI for 1 or 2 s. However, we are unaware of any research that has examined the combined effects of pressure-washing intensity and duration on NIS removal from shellfish. The present study expands on previous research by examining the interactive effects of pressure-washing intensity (2000 and 3000 PSI) and duration (10, 20, and 30 seconds) on NIS removal from string-cultured Pacific oysters. In addition, the research focuses on various key components of the biofouling community (i.e. NIS tunicates, crabs, shrimps, gastropods, as well as the entire epibiota community) as different species may be removed with different efficacies. Finally, the study assesses the impact of pressure washing on survival and shell condition of the shellfish themselves to determine the potential consequences of this physical removal technique.

Materials and methods

Field experiment

The study was conducted on a commercial Pacific oyster farm located in Clayoquot Sound (49°11′22.092″N; 125°55′34.803″W), on the west coast of VI (Figure 1), during June and July 2017. The farm produces oysters for the shucking market via a long-line, string-culture method, with 12–15 clusters

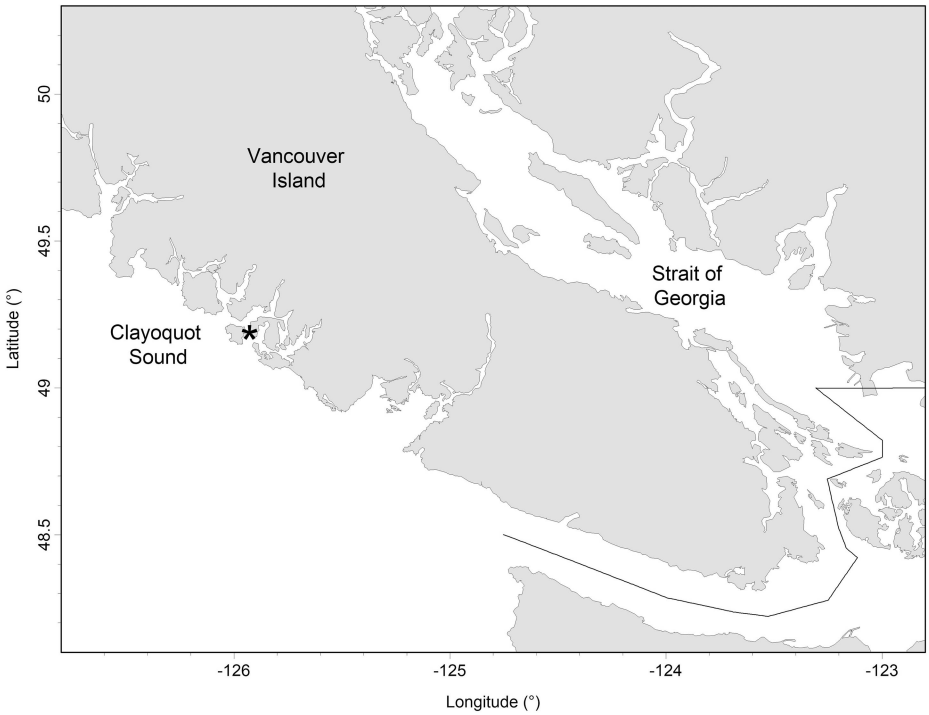

Figure 1. Map of shellfish farm study site on the west coast of Vancouver Island, British Columbia, Canada.

of oysters per 10-m drop line, the predominant form of oyster culture on the west coast of the island. Oysters produced via this method generally remain onsite for the duration of the grow-out period (2–3 years) and are typically rinsed only once during their life time, prior to transport to processing plants. The product is harvested, transported whole, with some level of associated biofouling, and shucked at processing facilities on the east coast of VI or the lower BC mainland as there are no processing plants on the west side of the island. Some NIS of interest have been previously found at this study site and include the European green crab, violet tunicate, golden-star tunicate, and carpet sea squirt (Curtis et al. 2015).

Extensive preliminary research on pressure washing oyster clusters guided experimental treatment levels and protocols. We initially tested a variety of pressure-washer nozzles (0, 15, 25, 45, 60°), intensities (1000, 2000, 3000, 4000 PSI), durations (5, 10, 20, 30, 40, 60 s), and distances from the tip of the pressure-washer wand to the oyster cluster (0.5, 1.0, 1.5, 2.0 m). A separation of 1.5 m was established as the minimum working distance where the operator could remain safe from flying dislodged material and water spray. At this distance, a 0° nozzle at 2000–3000 PSI was deemed to be most effective at removing NIS as water jets from larger-degree nozzles were too weak to remove NIS and higher PSIs resulted in oyster clusters being totally broken apart. Initially we hypothesized that commercial operators could not spend more than ~ 5 s per cluster, given the number of clusters that would require washing in a commercial setting, while consistently treating each cluster for a set time under 10 s was not scientifically reproducible. Thus, we chose 10 s per cluster to mimic the best

Table 1. The experimental Rinse treatments applied to oyster clusters with the codes used.

Code	Rinse treatment	Duration (s)
Control	None	0
Dunk	5 dunks	N/A
2000-10	2000 PSI	10
2000-20	2000 PSI	20
2000-30	2000 PSI	30
3000-10	3000 PSI	10
3000-20	3000 PSI	20
3000-30	3000 PSI	30

NIS removal that commercial growers could expect using a hand-held pressure washer and then tested two longer durations (20 and 30 s) to examine if extended times would increase NIS removal.

The experimental set-up was designed and executed under the conditions of a typical long-line, string-culture site along the west coast of VI. Many of these sites are remote with limited access to freshwater or a power source, making the implementation of some biofouling mitigation techniques difficult (e.g. pressurized heated brine or freshwater). With this in mind, we applied pressurized seawater to oyster clusters using a gas-powered, commercial, cold-water pressure washer (PE-4013HWPACAT 4000 PSI, BE Power Equipment, Abbotsford, BC, Canada) using a 0° nozzle head with seawater continuously supplied through a pump (Rule 2000 GPH, 12V bilge pump, Xylem Inc., Rye Brook, NY, USA). The water pressure was applied straight on at a distance of ~ 1.5 m from the oyster clusters. Pressure was monitored and maintained using a pressure gauge (BE pressure universal quick connect pressure gauge, BE Power Equipment).

Through preliminary research examining a range of intensities and durations (see above), pressure washing oysters at 2000 and 3000 PSI for 10, 20, and 30 s per cluster was determined to represent the best balance of equipment capability, efficacy of biofouling removal, and minimization of oyster loss/damage. Eight levels of experimental rinsing (henceforth termed Rinse) were applied that varied in the amount and duration of water pressure applied to the oyster clusters (i.e. pressure-time, Table 1). A "Control" treatment was established in order to determine the level of biofouling in the absence of any rinsing. The "Dunk" treatment represented how the shellfish aquaculture industry in BC may be interpreting the condition of licence obligation to rinse shellfish products prior to transport (DFO 2016). For this treatment, the oyster clusters were plunged and pulled out of the ocean (dunked) five times.

Individual oyster drop lines were pulled from the water using a crane and transported intact by boat to a nearby dock for experimental treatment. Individual clusters were cut from each drop line, leaving the rope within the cluster intact and with enough length to attach it to the experimental set-up. The top end of each cluster line was attached to a line that fed through a davit, with the end securely attached to a cleat. The bottom end

of the line was attached to a pulley system that was affixed to an anchor on the ground. The bottom line was pulled tightly through this pulley system until there was little to no movement of the entire set-up. This held oyster clusters in the same position as the culture lines and mimicked how a line could be treated as a whole.

The assignment of Rinse treatments to the oyster clusters was done using a stratified random block design in both June and July. Since biofouling density and species could be vertically stratified, we randomly assigned Rinse treatments prior to removal from the line within both shallow and deep oyster groups. Each Rinse treatment was randomly applied to one cluster within the first four clusters from the top and bottom of the drop lines, with clusters in the middle not used in the experiment. Further, since biofouling communities were unlikely uniform across the farm site, we blocked for treatment among the 12 replicates such that one replicate of each of the treatments was completed before the next replicate was collected.

After the Rinse treatments were applied, the clusters were individually placed in two or three 3-mil thick plastic bags and firmly closed to prevent any leakage or desiccation. The bags were put on sea ice within 1 hr and placed into commercial fish totes with sea ice for transport to the laboratory for processing. After transport, they were kept at −10 °C while the samples were processed fresh. During the sampling process the clusters were continuously monitored, remained cold, but the biofouling never froze. Keeping the samples fresh allowed for the identification of sessile biofouling and mobile invertebrates after processing as the organisms and their appendages were not degraded or destroyed through freezing.

Data collection, sample processing, sample sorting, and taxonomic analysis

Prior to applying the Rinse treatments, each cluster was assessed for the number of oysters per cluster, coverage of biofouling (rank), and visually scanned for the presence of various known NIS in BC including: violet tunicate, golden-star tunicate, Pacific transparent sea squirt (*Ciona savignyi* Herdman, 1882), carpet sea squirt, club tunicate (*Styela clava* Herdman, 1881), and the bryozoan *Bugula neritina* (Linnaeus, 1758). The biofouling coverage was ranked visually by eye as follows: 0, no visible fouling; 1, light fouling, covered in 1–2 very small patches of macrofouling, ≤ 15% surface area coverage; 2, considerable fouling, patchy macrofouling, 16–40%; and 3, extensive fouling, covering most of the visible surfaces of the oysters, 41–100% (Clarke Murray et al. 2013 adapted from Floerl et al. 2005). After Rinse treatments were applied, the shell condition of the oysters and a second biofouling coverage rank were recorded. Oyster-shell condition ranks were based on a quick scan of each cluster to assess the damage to shell valves on the majority of the oysters with the following categories: “Good”, no visible damage to the fringes of the valves or holes between the valves; “Fair”,

oysters had some damage to the fringes and edges of the valves, but no holes when closed; "Poor", oysters had extensive damage to the edges of the valves and holes when they were closed.

In the laboratory, each cluster of oysters was carefully removed from its bags, rinsed with freshwater, and broken apart. Individual oysters were inspected for NIS tunicates and crabs with organisms removed and separated if detected and then each oyster was scraped until all visible debris and epibionts were removed and rinsed again with freshwater. The rinse water, debris, and all the epibionts removed (including NIS tunicates and crabs) were filtered/rinsed through a pair of sieves. Excess water was removed and the remaining epibiont community, the NIS tunicates, and the crabs were each weighed separately. Total biofouling refers to all of the epibionts that were removed and weighed (i.e. sum of the filtrate, NIS tunicates, and crabs). Oyster clusters were also weighed before and after epibiont removal. All weight data were recorded to the nearest 0.1 g. Everything removed from the oysters and remaining on the sieve was retained and preserved in 95% ethanol for later sorting and taxonomic analyses. Sieves and all equipment used were thoroughly rinsed between each sample to avoid cross contamination.

The preserved samples were rinsed with distilled water and fractioned onto two sieve sizes (4000, 2000 μm). These fractions were visually inspected, separately, using a 43.2 × 35.6-cm tray marked with 3.8 × 3.8-cm gridlines and an LED lamp with a magnification (3X) lens. Mobile invertebrates were removed from the tray, separated, counted, and identified. Our focus was on known NIS crabs of the eastern Pacific (European green crab, Harris mud crab (*Rhithropanopeus harrisii* (Gould, 1841)), and Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853)) and NIS tunicates. All brachuryan and anomuran crustaceans (juveniles and adults) were separated and identified to the family level, using various taxonomic keys and references (Schmitt 1921; Hart 1935; Rice and Ingle 1975; Shanks 2001; Carlton 2007; Rice and Tsukimura 2007; Gonzales et al. 2009). If a specimen was suspected of being from the genera *Cancer*, *Hemigrapsus*, or *Carcinus*, or from the family Panopeidae, it was identified to species whenever possible. These genera and families of crabs are known NIS, or occupy similar niches as NIS crabs (McDonald et al. 2001). The carapace width (CW) of all crabs found was measured to the nearest 0.1 mm using *i*solution™ imaging software (Advanced Imaging Concepts Inc., Princeton, NJ, USA) when < 15-mm width or digital calipers when > 15-mm width and point and notch width were recorded when possible. Crabs from the family Pinnotheridae were not included in the total crab count. They are internal, commensal, or parasitic animals of bivalves (Schmitt 1921; Kuo et al. 2018) and it was not possible to decipher if experimental treatments affected their presence in the filtrate or if they came out of the oysters during sample transport, storage, or processing. Shrimps and gastropods were also counted

and identified to higher taxonomic groupings (henceforth crabs, shrimps, and gastropods will be collectively termed mobile invertebrates).

Data analyses

All biofouling and mobile invertebrate data were standardized as the weight and number of organisms (henceforth termed abundance) per gram of oyster, respectively, after all debris and biofouling organisms were removed. The crab abundance data were also split into two different size classes, small (≤ 10-mm CW) and large (> 10-mm CW), with three separate crab abundance variables being assessed (total, small, and large). Percent oyster loss describes the percent of individuals lost from the cluster due to pressure washing and was calculated by subtracting the number of oysters in a cluster post-Rinse treatment from the number pre-Rinse treatment, dividing by the latter, and multiplying the result by 100. This variable does not capture any information on the survival or quality of the oysters after the Rinse treatments were applied. The biofouling coverage data (ranks) were converted to the change in rank by subtracting the post-treatment rank from the pre-treatment rank, leading to 0, 1, or 2 changes in ranked coverage (hence 2 represents the greatest decrease in the coverage of biofouling). A total of eight oyster clusters were lost after Rinse treatments (five in June and three in July). An additional two samples were compromised and thus not used for the crab, shrimp, and gastropod data analyses.

The effect of the experimental treatments on total biofouling was analyzed through a full factorial analysis of variance with Rinse, Month, and Depth as main fixed effects, and all their interactions, with Block nested within Month as a random effect. Mobile species and NIS tunicate data were collected only in July, hence the effects of the experimental treatments on NIS tunicate weight and the abundance of mobile invertebrates were analyzed through another analysis of variance with Rinse and Depth as main fixed factors, their interaction, and a random Block effect. Significant main effects in both models were further analyzed through Tukey's HSD pair-wise comparisons. The assumptions of normality and equal variance were assessed through Normal quantile plots, scatter plots of the residuals versus predicted values of the model, and Levene's tests (Quinn and Keough 2002). If the data did not meet the model assumptions, they were transformed accordingly. The total biofouling and NIS tunicate data were log10(x+1) transformed and cube-root transformed, respectively. The crab, shrimp, and gastropod abundance data were log10(x+1), cube-root, and square-root transformed, respectively. The small and large crab abundance data, as well as the percent oyster loss data, did not meet the model assumptions after various transformations. These data were centered around the mean of each block of samples and the effects of Rinse and Depth were individually analyzed using the Kruskal-Wallis test (Chi-squared Approximation). Treatment means were substituted for missing data (see

Table 2. Organisms removed from oyster clusters after applying rinse treatments and identified to various taxonomic levels. These organisms do not encompass all those that were removed. NA = data analysis not performed.

Phylum	Level of taxonomic identification	Common name	Scientific name	Data analysis grouping
Mollusca	Class	Snails	Gastropoda	Gastropods
Mollusca	Species	Hooked slipper snail	*Crepidula adunca*	Gastropods
Mollusca	Order	Limpets	Patellogastropoda	Gastropods
Mollusca	Class	Chitons	Polyplacophora	NA
Arthropoda	Infraorder	Shrimps	Caridea	Shrimps
Arthropoda	Species	Black-clawed crab	*Lophopanopeus bellus*	Crabs
Arthropoda	Species	Pygmy rock crab	*Cancer oregonensis*	Crabs
Arthropoda	Species	Pubescent porcelain crab	*Pachycheles pubescens*	Crabs
Arthropoda	Family	Mud crabs	Panopeidae	Crabs
Arthropoda	Family	Porcelain crabs	Porcellinadae	Crabs
Arthropoda	Super family	Spider crabs	Majoidea	Crabs
Chordata	Species	Carpet sea squirt	*Didemnum vexillum*	NIS tunicates
Chordata	Species	Violet tunicate	*Botrylloides violaceus*	NIS tunicates
Chordata	Species	Golden-star tunicate	*Botryllus schlosseri*	NIS tunicates
Echinodermata	Class	Sea cucumbers	Holothuroidea	NA
Echinodermata	Class	Brittle stars	Ophiuroidea	NA
Echinodermata	Class	Sea urchins	Echinoidea	NA

above). In the case of percent oyster loss, data for both months were available, but the effects of Rinse and Depth were assessed individually per month. When an effect was significant, pair-wise comparisons were made using the Steel-Dwass all-pairs method.

The effect of Rinse and Depth on the change in the biofouling coverage rank and oyster-shell condition were analyzed through individual contingency tables (Chi-squared Approximation) with Cochran-Mantel-Haensznel tests that stratified the data by Block. The general association of categories was used to assess if a relationship between Rinse and biofouling coverage existed in at least one level of the Block effect. Treatment means were substituted for missing data (see above). If an effect was significant, a correspondence analysis was performed to compare levels of the effect. All analyses were carried out with JMP®, Version 14 (SAS Institute Inc., Cary, NC, USA). All reported values are mean ± SE.

Results

Total biofouling (abundance)

A list of all organisms recovered from the various treatments is given in Table 2. Rinse, Depth, Month, and the Month-Depth interaction all significantly affected the amount of total biofouling remaining (Table 3a). When compared to pressure-washed oysters, significantly more biofouling remained on the Control and Dunk oysters, with no significant difference between these two treatments (Figure 2A). There was a general trend towards increased intensity and duration resulting in less biofouling on the oysters, with 3000-20 and 3000-30 having significantly less biofouling than the Control, Dunk, 2000-10, and 3000-10 treatments (Figure 2A). Total biofouling remaining was significantly greater in July at both deeper (0.200 ± 0.013 g (g oyster)$^{-1}$) and

Table 3. (a) Analyses of variance of the effects of Rinse, Depth, Month (Total biofouling only), and Block on Total and NIS biofouling (g (g oyster)$^{-1}$) remaining on oyster clusters. (b) Contingency analyses, Chi-squared likelihood with Cochran-Mantel-Haenszel tests (C-M-H) of the effects of Rinse and Depth on the change in biofouling cover (rank) in June and July. *P*-values in bold are significant (<0.05).

(a)

Variable	Effect	MS_{num}	F_{df}	*P*
Total biofouling	Rinse	1.313	$52.231_{7, 322}$	**< 0.0001**
	Depth	0.800	$31.844_{1,322}$	**< 0.0001**
	Month	10.595	$277.743_{1,322}$	**< 0.0001**
	Rinse x Depth	0.038	$1.508_{7,322}$	> 0.05
	Month x Rinse	0.025	$0.978_{7,322}$	> 0.05
	Month x Depth	0.408	$16.218_{1,322}$	**< 0.0001**
	Month x Rinse x Depth	0.036	$1.417_{7,322}$	> 0.05
	Block[Month]	0.382	$1.518_{22, 322}$	> 0.05
	Error	0.025		
NIS tunicate (July)	Rinse	0.082	$3.066_{7,162}$	**< 0.01**
	Depth	0.020	$0.748_{1,162}$	> 0.05
	Rinse x Depth	0.005	$0.185_{7,162}$	> 0.05
	Block	0.067	$2.506_{11,162}$	**< 0.01**
	Error	0.027		

(b)

Month	Effect	χ^2_{df}; *P*	C-M-H χ^2_{df}; *P*
June	Rinse	137.261_{14}; **< 0.0001**	117.212_{14}; **< 0.0001**
	Depth	1.830_2; > 0.05	1.823_2; > 0.05
July	Rinse	124.843_{14}; **< 0.0001**	110.612_{14}; **< 0.0001**
	Depth	1.244_2; > 0.05	1.176_2; > 0.05

shallower (0.139 ± 0.008 g (g oyster)$^{-1}$) depths than in June (deeper (0.078 ± 0.004 g (g oyster)$^{-1}$) and shallower (0.074 ± 0.004 g (g oyster)$^{-1}$) depths).

Total biofouling (ranks)

Change in the coverage of total biofouling remaining was significantly affected by Rinse in both June and July, but not by Depth or their interaction (Table 3b). Correspondence analyses showed that in June, the Control and Dunk treatments grouped around 0 change in ranked coverage, while all other pressure-washing treatments centred around 1 and 2 changes (Figure 3A). In July, a third grouping appeared, as 3000-30 formed a separate, distinct group around 2 changes in ranked coverage, leaving all other pressure-washed treatments with the same groupings found in June (Figure 3B). These results indicate that dunking the oysters had no appreciable effect on the biofouling coverage when compared to the Control and, in general, pressure washing oysters removed a significant amount of the biofouling covering oyster clusters.

NIS tunicates

Didemnum vexillum was the dominant NIS tunicate found on the oyster clusters compared to both botryllid tunicate species that had minimal coverage. Rinse and Block significantly affected the amount of NIS tunicates

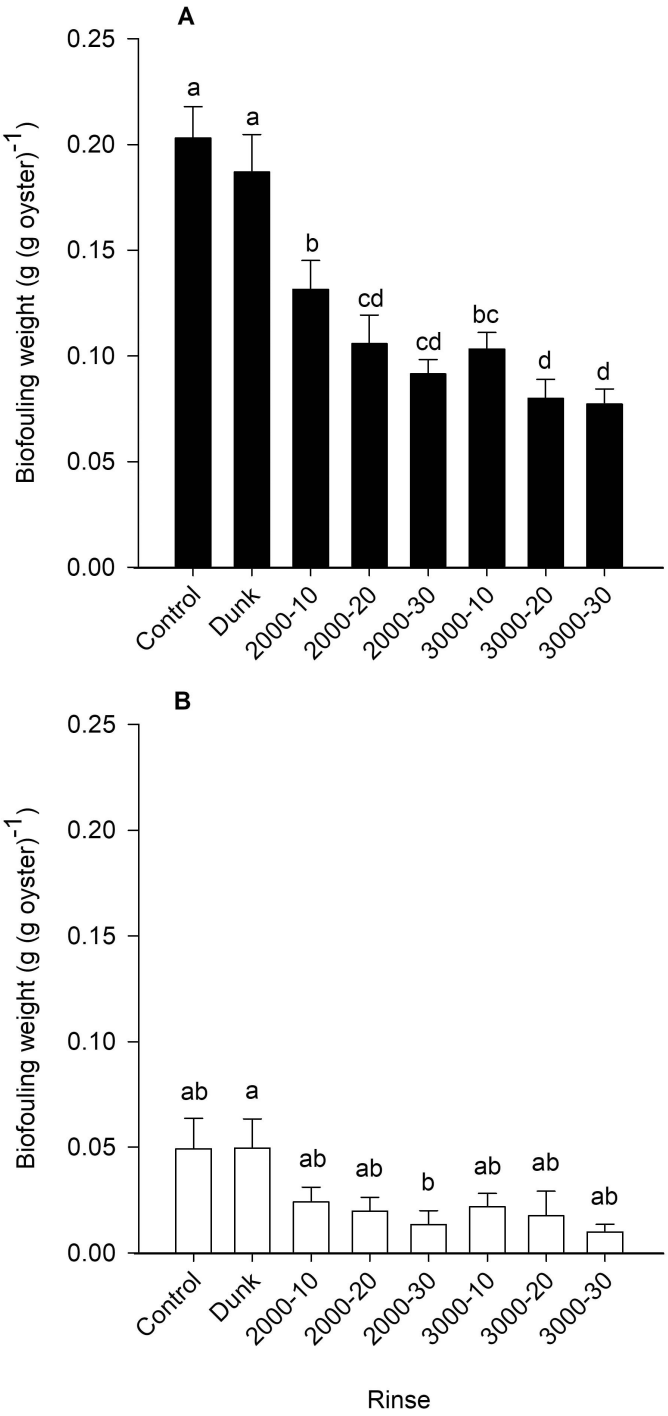

Figure 2. Mean + SE (A) total (n = 48) and (B) non-indigenous species (NIS) tunicate (n = 24) biofouling weight (g (g oyster)$^{-1}$) remaining on oyster clusters after experimental rinse treatments were applied (see Table 1 for treatment codes). Treatments with different letters are significantly different ($P < 0.05$, Tukey's test).

remaining on the oysters (Table 3a). Only one pairwise comparison among the Rinse treatments was significant, with 2000-30 having significantly less NIS tunicate weight remaining than the Dunk treatment (Figure 2B). There was significantly less NIS tunicate weight remaining in the first block of samples (0.011 ± 0.010 g (g oyster)$^{-1}$) when compared to the fourth (0.053 ± 0.020 g (g oyster)$^{-1}$) or eighth (0.040 ± 0.011 g (g oyster)$^{-1}$) blocks.

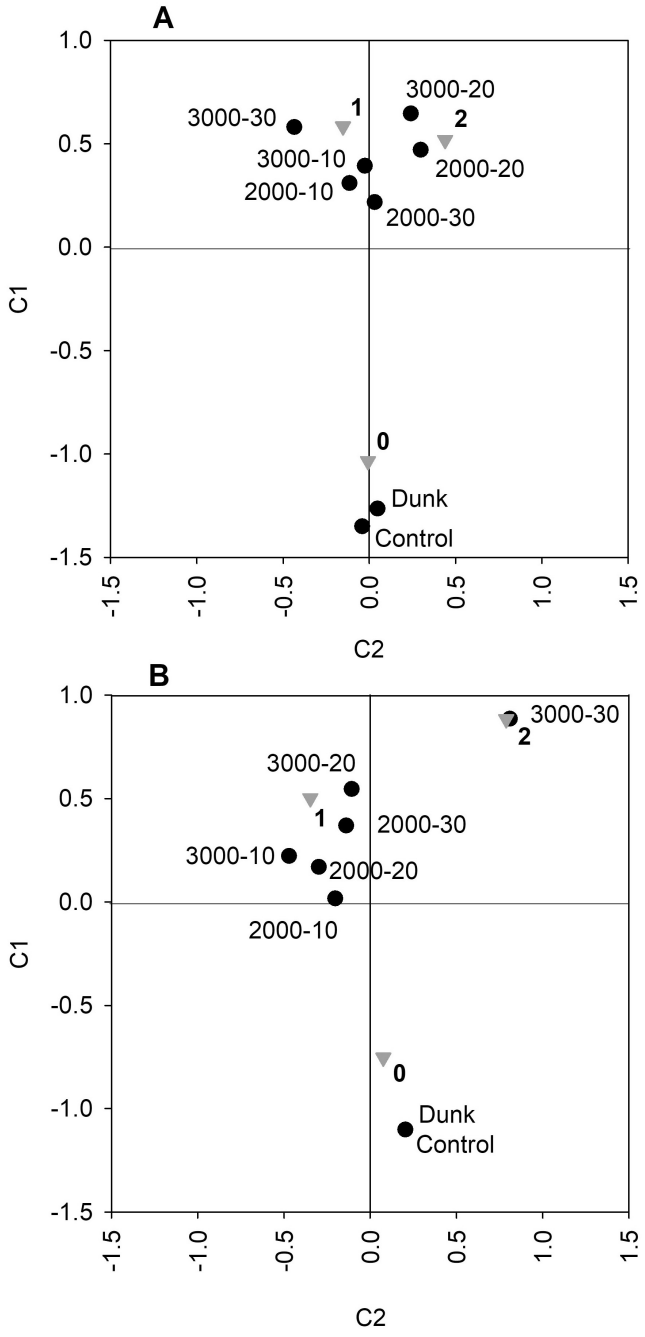

Figure 3. Correspondence analysis of the change in rank biofouling coverage after experimental rinse treatments were applied in (A) June (n = 24) and (B) July (n = 24) ($P < 0.05$ for both months; contingency analyses with Cochran-Mantel-Haenszel tests). Grey triangles represent changes in ranked coverage with 0 = no change in coverage, 1 = one rank change, and 2 = two rank changes (i.e. greatest decrease) (see Table 1 for treatment codes).

Mobile invertebrates

None of the experimental Rinse treatments removed 100% of the mobile invertebrates assessed in this study. Six species of crab were present on the oysters, but were dominated by black-clawed crab (*Lophopanopeus bellus* (Stimpson, 1860)), pygmy rock crab (*Cancer oregonensis* (Dana, 1852)), and pubescent porcelain crab (*Pachycheles pubescens* Holmes, 1900), in order of abundance. Specimens identified simply to family belonged to Panopeidae,

Table 4. Analyses of variance of the effects of Rinse, Depth, and Block on remaining crab, shrimp, and gastropod abundances (individuals (g oyster)$^{-1}$) on oyster clusters. *P*-values in bold are significant (< 0.05).

Variable	Effect	MS_{num}	F_{df}	*P*
Crabs	Rinse	$1.468E^{-8}$	$12.160_{7,160}$	**< 0.001**
	Depth	$1.003E^{-10}$	$0.083_{1,160}$	> 0.05
	Rinse x Depth	$3.062E^{-9}$	$2.537_{7,160}$	**< 0.05**
	Block	$1.733E^{-9}$	$1.435_{11,160}$	> 0.05
	Error	$1.207E^{-9}$		
Shrimps	Rinse	0.052	$17.017_{7,160}$	**< 0.0001**
	Depth	0.003	$1.080_{1,160}$	> 0.05
	Rinse x Depth	0.005	$1.522_{7,160}$	> 0.05
	Block	0.006	$2.072_{11,160}$	**< 0.05**
	Error	0.003		
Gastropods	Rinse	$8.933E^{-4}$	$1.600_{7,160}$	> 0.05
	Depth	$4.694\ E^{-4}$	$8.300_{1,160}$	**< 0.01**
	Rinse x Depth	$1.223E^{-4}$	$2.100_{7,160}$	**< 0.05**
	Block	$2.922\ E^{-5}$	$0.520_{11,160}$	> 0.05
	Error	$5.663\ E^{-4}$		

Table 5. Kruskal-Wallis analyses, Chi-squared approximation, of the effects of Rinse and Depth on small (≤ 10-mm carapace width) and large (> 10-mm) crabs remaining on oyster clusters (individuals (g oyster)$^{-1}$). *P*-values in bold are significant (< 0.05).

Size class	Effect	χ^2_{df}	*P*
Small	Rinse	48.619_7	**< 0.0001**
	Depth	0.032_1	> 0.05
Large	Rinse	18.501_7	**< 0.05**
	Depth	2.224_1	> 0.05

Porcellinadae, and Majoidea, in order of abundance. The abundance of all crabs (of all sizes) was significantly affected by Rinse and the Rinse-Depth interaction (Table 4, Figure 4A). Rinse had no significant effect on the total number of crabs remaining on shallow oysters, but it did have a significant effect on those grown in deeper water. Significantly fewer crabs remained in all the pressure-washed treatments from deeper water when compared to the Control, but there were no significant differences in those crabs remaining among all the pressure-washed treatments nor between the Control and Dunk treatments (Figure 4A). In addition, there were significantly fewer crabs in all the pressure-washed treatments (except 2000-20) than in the Dunk treatment.

Both small and large crabs were significantly affected by Rinse, but not by Depth (Table 5). There were significantly fewer small crabs in all the pressure-washing treatments than in the Control and Dunk treatments (except for the 2000-10 versus Dunk comparison) and no significant difference between Control and Dunk (Figure 5). For large crabs, significantly fewer individuals were found in the 2000-20 and 2000-30 treatments than in the Control, with no other significant pairwise comparisons (Figure 5).

The abundance of shrimp was significantly affected by Rinse and Block (Table 4). Tukey post-hoc analysis revealed that all pressure-washing treatments and the Dunk treatment significantly reduced the number of shrimp relative to the Control (Figure 4B). The 2000-30 and 3000-30 treatments

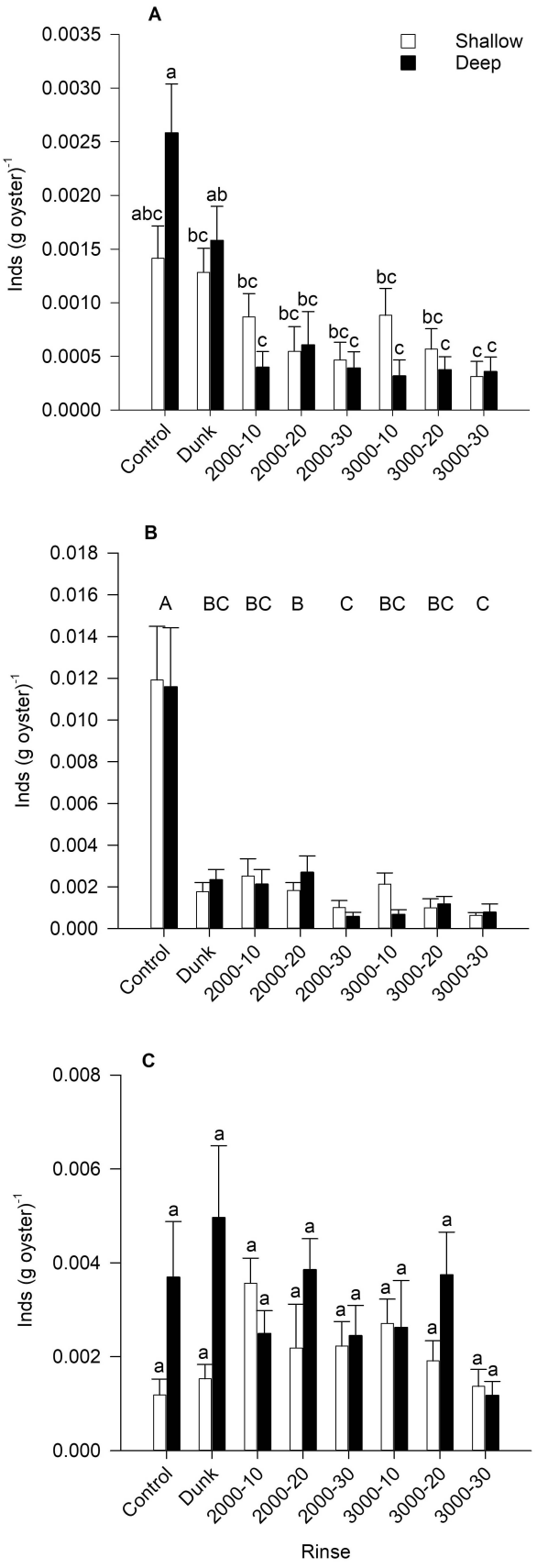

Figure 4. Mean + SE number of (A) crabs, (B) shrimps, and (C) gastropods remaining on oyster clusters (individuals (g oyster)$^{-1}$) in response to experimental rinse treatments and depth (n = 24 for A-C). Treatments with different letters are significantly different (P < 0.05, Tukey's test). Upper-case letters apply to the effects of the experimental rinse treatments alone and lower-case letters apply to the interaction between rinse treatment and depth.

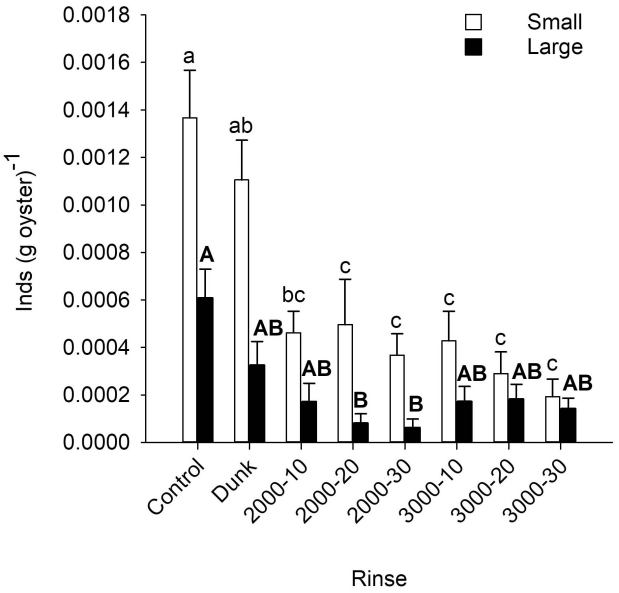

Figure 5. Mean + SE number of small (≤ 10-mm carapace width) and big (> 10-mm) crabs remaining on oyster clusters (individuals (g oyster)$^{-1}$) after exposure to experimental rinse treatments (n = 24 for both size classes). Treatments with different letters are significantly different ($P < 0.05$, Steel-Dwass All Pairs). Lower-case and upper-case letters represent significant differences among the small and large crabs, respectively.

had significantly fewer shrimp than the 2000-20 one (Figure 4B). While the Block effect was significant in the main ANOVA, pairwise comparisons did not detect any significant differences.

The abundance of gastropods was significantly affected by Depth (Table 4), with significantly more gastropods remaining on oysters from deeper clusters (0.003 ± 0.0003 individuals (g oyster)$^{-1}$) than shallower ones (0.002 ± 0.0002 individuals (g oyster)$^{-1}$). The interaction of Rinse and Depth was also significant (Table 4), however, Tukey post-hoc analysis revealed no significant pairwise differences between treatments.

Oyster loss and shell condition

Percent oyster loss was significantly affected by Rinse in both June and July, but not by Depth (Table 6A). In June, the 3000-20 and 3000-30 treatments led to significantly greater percent oyster loss when compared to the Control and Dunk treatments (Figure 6A). Although a Kruskal-Wallis test found that Rinse had a significant effect on oyster loss in July and trends appeared similar to June, Steel-Dwass pairwise comparisons did not reveal any significant differences among treatments in July (Figure 6A).

Oyster-shell condition was significantly affected by Rinse in July, but not in June, and was not significantly affected by Depth in either month (Table 6B). Control, Dunk, 2000-10, and 2000-30 formed a tight grouping predominantly around Good condition, 3000-10 and 3000-20 fell between Fair and Good, 3000-30 fell between Fair and Poor condition while 2000-20 corresponded with all three oyster-shell conditions (Figure 6B).

Table 6. (a) Kruskal-Wallis, Chi-squared approximation, analyses of the effects of Rinse and Depth on percent oyster loss in June and July. (b) Contingency analyses, Chi-square likelihood with Cochran-Mantel-Haenszel tests (C-M-H) of the effects of Rinse and Depth on oyster-shell condition in June and July. *P*-values in bold are significant (< 0.05).

(a)

Month	Effect	χ^2_{df}	*P*
June	Rinse	25.967_7	**< 0.001**
	Depth	0.011_1	> 0.05
July	Rinse	18.275_7	**< 0.05**
	Depth	1.049_1	> 0.05

(b)

Month	Effect	χ^2_{df}; *P*	C-M-H χ^2_{df}; *P*
June	Rinse	19.807_{14}; > 0.05	16.646_{14}; > 0.05
	Depth	1.321_2; > 0.05	1.523_2; > 0.05
July	Rinse	43.830_{14}; **< 0.0001**	44.709_{14}; **< 0.0001**
	Depth	0.002_2; > 0.05	0.001_2; > 0.05

Discussion

Pressure washing significantly reduced the total biofouling (seen with both the weight and rank coverage data) in comparison to Control and Dunk treatments, however, it was not 100% effective, as 38–65% of the total biofouling still remained on the oysters after treatment. Increased duration resulted in higher efficiency of removal, regardless of the pressure applied, with the 3000-20 and 3000-30 treatments resulting in significantly less total biofouling remaining than the 2000-10 and 3000-10 ones. In addition, the results also showed that applying lower pressure for longer durations (2000-20 or 2000-30) resulted in statistically the same amount of total biofouling remaining as for higher pressure for shorter durations (3000-10). These results fall within the pressure-washing efficacies reported by Paetzold et al. (2012) for removing epifauna from mussels (*Mytilus* sp.) with 700 PSI for 10 s and by Forrest and Blakemore (2006) for removing Asian kelp gametophytes (*Undaria*) from tuatua (*Paphies subtriangulata* (W. Wood, 1828) with 1000–3000 PSI for 1–2 s (i.e. ranges of 60–70% removal or 30–40% remaining).

Pressure washing was not as effective for NIS tunicates as it was for total biofouling, as no Rinse treatment significantly reduced NIS tunicate weight compared to the Control and only one treatment (2000-30) significantly reduced it in relation to the Dunk treatment (but only by ~ 30%). Pressure washing has been shown to be a rather effective means at removing some species of tunicates, particularly botryllid tunicates fouling cultured shellfish species (Arens et al. 2011; Paetzold et al. 2012), but findings may be context dependent with different efficiencies both among NIS tunicate species and the aquaculture product they are fouling (e.g. mussels versus oysters). Thus, the relative ineffectiveness in the present study may be due to: (1) the dominant tunicate present, *D. vexillum*, which can become quite thick and convoluted, with multiple layers and folds within crevices and between shell

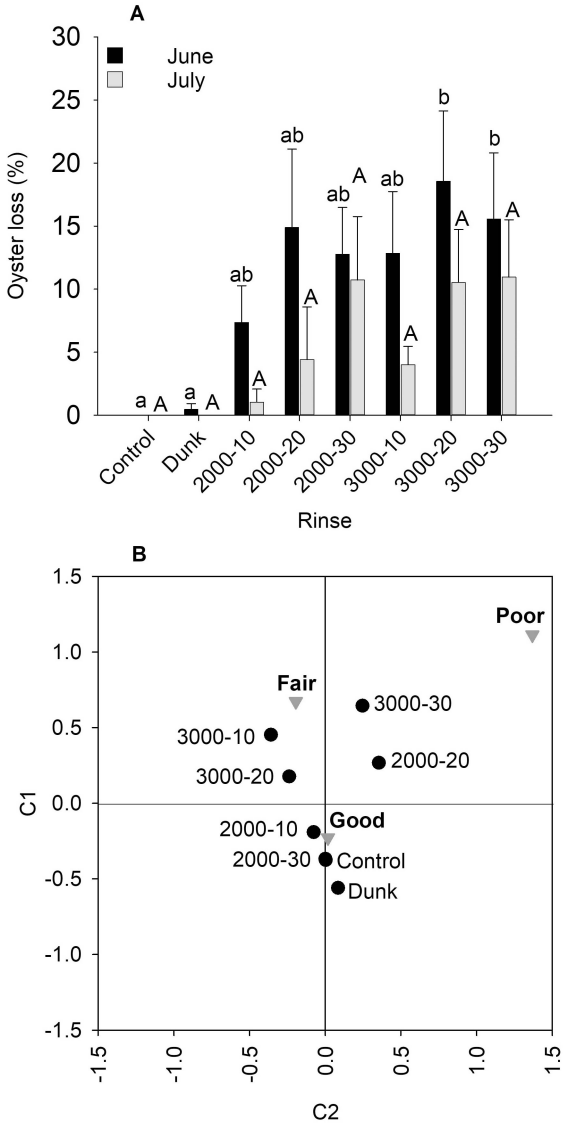

Figure 6. (A) Mean + SE percentage of oysters lost after exposure to experimental rinse treatments in June and July (n = 24 for both months). Treatments with different letters are significantly different ($P < 0.05$, Steel-Dwass All Pairs). Lower-case and upper-case letters represent significant differences in June and July, respectively. (B) Correspondence analysis of the effect of rinse treatment on oyster-shell condition in June ($P < 0.05$, contingency analysis with Cochran-Mantel-Haenszel tests; n = 24). Grey triangles represent oyster-shell condition after treatment application (Good, Fair, and Poor).

valves, making it less susceptible to control or removal measures (Walters and Wethey 1996; Kott 2002; Switzer et al. 2011), (2) the shellfish species used (Pacific oysters, which have a much more rugous and convoluted shell surface than blue mussels), or (3) different pressure-washing machinery.

Pressurized-seawater treatments resulted in 17–34%, 14–34%, 23–28%, 6–20%, and 52–127% of the total crabs, small crabs, large crabs, shrimps, and gastropods remaining on the oysters, respectively, when compared to doing nothing (Control), a reduction that was generally significant. These ranges of efficacy, however, did not usually translate into significant differences among the various Rinse treatments as there were no significant pairwise differences in the abundances of total crabs, large crabs, small

crabs, and gastropods remaining among the various Rinse treatments (Figures 4 and 5, Table 4) with the only exception being shrimps, where 2000-30 and 3000-30 treatments resulted in significantly fewer individuals remaining than the 2000-20 treatment. Thus, pressure washing consistently resulted in individuals of numerous taxa remaining associated with the oysters and therefore available for movement away from the farm site. It is unlikely that any single mitigation measure used at the shellfish farm would reduce this to no remaining individuals of any taxa, due to species-specific behaviours (see below), such that other control points would need to be invoked to reduce the overall risk of secondary spread of NIS.

No combination of pressure or duration resulted in complete removal of sessile or mobile biofouling, which may be at least partially explained by the complex, three-dimensional habitat Pacific oysters form, especially when grown in clusters, and the behaviour of crabs, shrimps, and gastropods. Even as newly settled juveniles, many crab species actively choose complex habitats, are camouflaged, or directly avoid predators and hostile environmental conditions by hiding in interstitial spaces such as those created between oysters (Richards 1992; Pardieck et al. 1999; Simonik and Henry 2014). By actively seeking such protection, some crabs would not be vulnerable to the pressure-washing treatments employed in the present study. The only way to remove hiding spaces for the crabs would be to completely break oyster clusters apart prior to rinsing, a practice that would be very time consuming and unlikely to be employed by shellfish growers. In contrast to crabs, the number of shrimp remaining associated with oysters after treatment, including Dunk, were significantly reduced relative to the Control, which also can be attributed to their behaviour. When exposed to strong external stimuli, caridean shrimp generally use tail flipping (Arnott et al. 1998) as an escape response, such that when clusters are manipulated these species flee rather than seek protection deeper in the oyster clusters. For taxa such as gastropods, individuals offered more protection by the oyster clusters were less vulnerable to pressure-washing treatments than those that would be more exposed and thus more easily removed.

Pressure washing at the farm site can significantly reduce the amount of sessile biofouling and mobile invertebrates on shellfish (propagule pressure) when compared to doing nothing (control) or even the purported industry practice of dunking. While not 100% effective, with efficacy differing among taxonomic groups, it does represent an important control point for mitigating secondary spread by NIS, but also highlights that other control points are needed to ensure propagules are not released to new environments. Curtis et al. (2015) provide examples of other control points related to shellfish movements in BC and some of these could be imposed at the farm site, during transport, or at the processing facilities. At the farm for example,

dual treatments of heat and acids have been suggested as a way to reduce *Undaria* propagules in oyster (*Ostrea angasi* GB Sowerby II, 1871) culture in Australia (Sievers et al. 2019). Similarly, desiccation and heat treatment can be used at either the harvest or processing site to reduce NIS crab propagules (Best et al. 2014; Hopkins et al. 2016). Further, other downstream control points exist. For example, treating the effluent or waste water (Veiga et al. 1994) and fouled shell discards associated with shellfish processing may be another practical solution. Further research is required to examine the efficacy of other mitigation strategies and potential control points in order to reduce risks of movement of NIS on shellfish. Experiments determining the efficacy of pressure washing and other potential methods (e.g. tumbling with water jets and manual scrubbing) for NIS removal on tray- or bag-cultured single oysters and bottom-cultured oysters are also needed. Economic assessments are required to determine the commercial viability of all of these NIS-removal methods.

In conclusion, none of the pressure-washing intensity/duration combinations tested in this study were effective at removing 100% of the biofouling or mobile invertebrates assessed, thus leaving at least some (perhaps many) individuals associated with Pacific oysters to be spread via shellfish movements. Further, our results suggest that repeated dunking of shellfish product will not significantly reduce sessile biofouling or mobile invertebrate species (except shrimp) in relation to doing nothing (study control). Thus, the purported practice of dunking shellfish in seawater by industry appears to be less effective than the intended or desired reduction in propagule pressure of NIS related to oyster movements. Pressure washing as a mitigation strategy does have some negative consequences for the cultured species themselves as oyster loss from the clusters and shell damage was observed during the experimental treatments. Physical loss of oysters could be prevented by placing a large draped net behind the oyster strings to catch any individuals blown off by the pressure washing. Further research is required to assess the role of shell damage in short-term oyster survival as string-cultured oysters are likely only washed once (immediately prior to transport to processing plants), are subject to relatively short transport times, and are destined for the shucked (not half-shell) market where shell condition is less important.

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