A review of two sampling methods for detecting Surf Smelt (*Hypomesus pretiosus*) spawn on intertidal beaches for marine ecosystem conservation and restoration.

Shaffer[1*] J. Anne, Kirsten A Simonsen[1], Dan Penttila[2], and Ellie Stevenson[1] , Seren Weber[1] and David Harvey[1]

[1]Coastal Watershed Institute (CWI)
P.O Box 266, Port Angeles, WA 98362
360.461.0799; Anne.shaffer@coastalwatershedinstitute.org
*Study contact

[2]Salish Sea Biological, 5108 Kingsway, Anacortes, WA 98221, USA. Email: depenttila@fidalgo.net

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Abstract

Surf Smelt (*Hypomesus pretiosus*), a critical component to northeast Pacific marine ecosystems, spawn along select intertidal beaches. In this study we compared two established egg-winnowing techniques currently used to detect surf smelt eggs along a series of beaches, including those used for long term monitoring for ecosystem restoration associated with large-scale dam removals. Our results confirm that surf smelt spawning along north Salish sea beaches is variable with month and site, and that surf smelt spawning continues to extend along newly restored beaches. There were no statistical differences were found in the number or stage of eggs identified between the two techniques. We conclude that both methods are valid tools for detecting and monitoring surf smelt spawning on Salish Sea beaches, and that winnowing method used therefore should be decided based on study goals. The newer (Vortex) method, which has recently been adopted by WDFW as the standard monitoring tool for future forage fish spawn mapping, is much more gear and time intensive, but relatively mechanistic and requires little technique or training. The original (Original) method is faster, less expensive, and has been used for decades of monitoring across the Salish Sea, but may take more training to learn and implement consistently across field staff and citizen monitoring efforts.

As standard monitoring techniques change it is important to define a data conversion to standardize data so changes observed in field monitoring can be accurately interpreted as habitat change, and not an artifact of a change in sampling technique. Our study determined that linear regression analysis is a suitable tool for calculating a conversion factor if multiple techniques are used during a long-term study, or if results are going to be compared across studies that use both of Original and Vortex techniques. We recommend at least one year of paired sampling should be conducted to calculate a regression factor for conversion and comparison purposes.

Brief summary

Surf Smelt (*Hypomesus pretiosus*) belong to a guild of forage fish that spawn on intertidal beaches, which is used as a proxy for ecosystem function and a guide for shoreline conservation and restoration management. This study compares, for the first time, two standard methods used to detect surf smelt eggs, and provides guidance on their use as a conservation tool.

Introduction

Forage fish serve as an important link between autotrophic and heterotrophic systems and therefore are a cornerstone of marine ecosystems as well as primary and secondary commercial fisheries worldwide. Pikitch *et al*. (2014) estimated the global catch value of forage fisheries at US $5.6 billion, while fisheries supported by forage fish were more than twice as valuable (US $11.3 billion). Declining trends in forage fish populations worldwide have therefore become a global concern. Population growth and overfishing contribute to forage fish population declines (Greene *et al.* 2015), though due to complex life histories, impact source and response is challenging to define for forage fish (Engelhard *et al*., 2014). There is growing concern about ecosystem scale ramifications of rapid loss of forage fish stocks. Much of the emerging dialog on forage fish conservation has focused on fishery management (The Pew Charitable Trusts 2015; National Oceanic and Atmospheric Administration 2016).

In the northeast Pacific, a number of forage fish species including Surf Smelt (*Hypomesus pretiosus*), Herring (*Clupea pallasi*), Eulachon (*Thaleichthys pacificus*) and Sand Lance (*Ammodytes hexapterus*) have very specific dependence on the nearshore for migration, rearing, spawning, and feeding. For example, Eulachon, Surf Smelt, and Sand Lance are documented to have very specific seasonal grain size requirements for intertidal spawning (Reeves *et al*. 1989; Penttila 2007; Martin 2014). Surf Smelt prefer a mixed sand gravel substrate, while Sand Lance prefer mixed sandy beaches (Penttila, 2007).

Over the last two hundred years, shorelines crucial to forage fish migration and possibly spawning have been degraded through development (Martin 2014; Pilkey and Cooper 2014). In particular disruption of nearshore hydrodynamic and sediment processes -such as large scale dams and shoreline armoring- can result in major declines in ecosystem function, including for intertidal beach spawning forage fish (Bottom *et al.* 2005; Rice 2006; Dugan *et al*. 2008; Parks *et al.* 2013; Toft *et al*. 2013; Parks *et al* 2015; Dethier *et al.* 2016).

In response to the importance of intertidal habitat for forage fish spawning, and the risk to this ecosystem function from development, the Washington State Department of Fish and Wildlife (WDFW) has managed critical forage fish spawning habitat for decades, based on state laws that define habitat management actions to conserve documented spawning zones (WDFW 2019). To achieve these state mandates, WDFW developed and implemented standard sampling and winnowing techniques to detect and quantify forage fish spawning in northeast Pacific shorelines beginning in the 1980s (Moulton and Penttila 2001). These methods have been used extensively throughout the Pacific Northwest for over 40 years to define the presence and extent of forage fish spawn along Salish Sea shorelines. These methods are an invaluable tool for detecting, studying, preserving, and restoring surf smelt spawning throughout the region, and data generated from them are the basis for a number of important long-term monitoring studies (Shaffer 2017; Shaffer *et al* 2017). In addition, it is critical to understand how the two methods compare to each other.

More recently, a new method has been developed by Dionne (2016), known as the "Vortex method'.   This method has been adopted in the last five years by WDFW and is now the standard method for detecting intertidal forage fish spawn. Dionne (2016) reports differences in egg detections between the two methods, but provides not quantitative comparison. Given the extent (both geographically and temporally) that the original Moulton and Penttila 2001 method has been used to map forage fish spawning, it is critical to understand the relationship between these two methods. Long term studies will continue and methods need to be contemporary so results can be compared to other work occurring at the same time. There is, therefore, a need and an interest in transitioning methods used in long-term studies so that they are consistent with current methods, and a need standardize data collection methods so that current, future, and historic data can be compared. This requires a confirmation that new sampling techniques are compatible with, and consistently comparable to, original spawn detection methods. In this study we used our long-term annual sampling of beaches along the central Strait of Juan de Fuca as an opportunity to compare these methods and answer the following two questions:

1. Do the “Original” (O) (Moulton and Penttila 2001) and “Vortex” (V) (Dionne 2016) winnowing methods result in statistically different egg counts for surf smelt spawn assessment?
2. Can a relationship between the two methods be developed allowing the conversion of results between the two methods in future long-term monitoring studies?

From this field study we can then decide the best path to continue monitoring studies, as well as link historic and long-term observations to future efforts to best conserve and restore critical forage fish spawning habitat in the future.

Methods and Materials

Six long-term forage fish monitoring sites were used in this study. Sites sampled were in varied state of alteration, and included armored beaches, unarmored beaches, and beaches newly restoring due to sediment delivery from Elwha dam removals (see Shaffer *et al* 2017 for an overview of the nearshore Elwha project). Samples were collected in the field using the standard field technique developed by Moulton and Penttila (2001) and also used in Dionne (2016). Overall linear distance of each site sampled ranged from 90m to over 2000m (Figure 1; Tables 1 and 2).

Paired samples were taken along a series of 30m transects along each site. The number of transects varied with beach so that all beaches were sampled with approximately the same intensity. For each 30m transect, a hand scoop was used collect the top 2-3cm of sediment along the last high tide line along the upper one third of the beach (approximately Mean High Water Line Mark (MHWM) or higher elevation). For each sample, sediment along each transect was collected in two identically sized plastic bags, each with a duplicate tag recording sample location number date and time. All information was also recorded on data sheets.

Paired samples were winnowed using one of two methods.

i. Original processing method (O)

Per Moulton and Penttila 2001, each sample was rinsed with a hose through size 2 and 0.5mm mesh screens. Sediment from the smaller mesh was transferred to a plastic dish tub with a shallow covering of water and agitated for 1-2 minutes to bring the eggs to the surface. The sediment was then skimmed from the surface and collected in 16oz plastic jars with the sample tags. This process was repeated two more times on the remainder of the sample and collected in the same jar and then preserved with Stockard's solution. All equipment was thoroughly rinsed between samples to prevent cross contamination. Processed samples were analyzed in the lab as described below.

ii. Vortex processing methods (V):

Per Dionne 2016, each sample was rinsed with a hose through size 2 and 0.5mm mesh screens. The sediment was transferred from the 0.5mm mesh sieve to a plastic dish tub and the sieve was placed under the blue concentrator bowl. The adjustable valve on the blue concentrator bowl was opened half way and the pump turned on. Up to 60oz of the sediment was added to the bowl from the 0.5mm mesh sieve with the rubber spatula. The water flow was then adjusted so that the water was 2cm from the top of the bowl. Sediment was then stirred with the plastic spoon with straight lines from the middle to the outer wall of the bowl for 1-3 minutes. The mesh sieve was removed from under the blue bowl and all material was washed into a 16oz plastic jar with a wash bottle and preserved with Stockard's solution. Vortex processed samples were then analyzed in the lab as described below.

All samples were given unique ID numbers that were keyed to winnowing methods. These ID method keys were not forwarded with the samples for lab processing. Lab processing was therefore done blind, with no identification of the winnow method used to generate the sample throughout the lab processing. Processed samples were examined under a dissecting microscope and all eggs identified, counted, and life-history stage recorded.

As documented in earlier studies (Parks *et al* 2013), egg counts were highly variable in both space and time and therefore, as is often the case in ecological count data, consisted of a large number of zeros (Zuur *et al*. 2009). Therefore, nonparametric tests were most appropriate to test for differences in the data due to the non-normal distribution of the data and the small sample size. A Kruskal-Wallis test was used to test for differences between sampling method (Original or Vortex), month (May or June), and site, using the stats package (version 3.5.1) in R (R Development Core Team 2014). To further reduce bias that might be associated with the high number of zeros in the data (Table 2), a second test was run on only those sites with a total $n > 10$, to ensure the analysis included a large enough sample size to detect differences between the two techniques. For this analysis, the sites Cline Spit and Twins were included, as they were the only sites out of the six sampled that fit the criteria for a robust analysis.

A Model II linear regression using ordinary least squares (OLS) was calculated comparing the number of eggs collected using each method to determine a conversion factor between methodologies. To do this, the numbers of eggs collected from all six sampling sites were used. Eggs collected using the Vortex method were modeled as a factor of eggs collected using the Original method to determine if a significant relationship existed between the two methods. A hypothesis test was then run to test if the slope of the regression was significantly different from 1 to further test whether the two methods were significantly different from each other.

Results

The number of surf smelt eggs collected varied with both site and month. A few sites had no eggs, while others had consistently high egg abundances. For example, nearly three times as many eggs were collected at Cline Spit than at Twins (Figure 2), and more eggs were collected in May than in June (Table 1 ,2 ; Figure 3). Egg stage and brood size also varied with site (Table 3).

Comparing the two winnowing methods, results of the Kruskal-Wallis test showed that there were significant differences in the number of eggs collected between site ($p < 0.0001$) and month ($p = 0.00048$) (Table 4). While the Original method results had a higher range of values, there were no significant differences observed between methods ($p > 0.05$). Therefore, all differences observed in collection were due to location and time. For the reduced model examining only Cline Spit and Twins, there was a significant difference in the number of eggs collected between site ($p < 0.0001$), but not month ($p = 0.051$) (Table 4). The reduced model also found no significant differences observed between method ($p > 0.05$) (Table 4).

The Model II linear regression fit between the two different winnowing methods was significant, showing a positive relationship (Figure 4) with the following formula:

$$Eggs_V = 1.095*Eggs_O + 2.45$$

where $Eggs_V$ is the number of eggs collected using the Vortex method, and $Eggs_O$ is the number of eggs collected using the Original method. The final regression had an $r^2 = 0.86$, with a slope of 47.6 degrees, indicating a good fit to the data. Hypothesis testing found that the slope was not significantly different from one ($p > 0.05$), further indicating that the two methodologies are not collecting significantly different numbers of eggs.

Discussion

Surf smelt spawn abundance and distribution varied substantially between site and sampling month. Such variability in spawn abundance is well documented for this region of the northeast Pacific (Quinn *et al* 2012, Shaffer *et al* 2012, Parks *et al* 2013, Wefferling 2014). Egg mortality was high in areas with high egg abundance and likely due to lack of shade, as well as shoreline armoring at the site (Rice 2006; Penttila 2007).

The two winnowing methods yielded similar results. Quantitative analysis revealed that, even when the analysis data set was reduced to those sites containing the largest number of eggs, we found little evidence of differences between winnowing methods in the detection or counts when present of eggs. In particular, there was no significant difference between Original and Vortex methods in the number of eggs counted, egg development stage, brood count, or percent dead between the two methods (Table 3). From these results we conclude that the spatial and temporal variability in habitat use exceeds the variability in winnowing methods used to detect eggs. We further conclude that a simple regression can be generated to allow conversion of data between the two methods-critical for long term monitoring data.

However, other non- quantitative factors were different between the two methods. Specifically, the Vortex method, which includes the use of pumps and a recirculating tub, is much more gear intensive method. As a result it takes, on average, twice the amount of time as the Original method for sample processing.

Based on these results we conclude that the Original and Vortex winnowing methods are both valid and useful tools for detecting and monitoring surf smelt egg spawning on intertidal beaches, and recommend their use as a standard monitoring technique. In particular, as the Vortex (Dionne 2016) method has been adopted by the WDFW as the standard protocol for forage fish spawn study.in general we recommend using it in the future. However, other factors in addition to the number of eggs extracted from a sample are important to consider when deciding which method to use. Specific considerations when deciding which method to use include:

1. The goal of the sampling (specifically whether the intent of the sampling is simple egg detection or to quantify the spawn abundance).
2. Cost investment for equipment, staff time, and resources for sample work. The Original method yields statistically similar results, and is a much simpler technique

for detecting forage fish eggs. The Original method may therefore be most appropriate for initial field assessments/projects with limited budgets.

Given the decades of study that have been conducted using the Original method, and the desire by managers to use the Vortex method in the future in Washington State, there needs to be a bridge to allow comparison of data from the two winnowing methods. This work illustrates that a straight forward correlation can be generated to link the two methods. Given the high variability in forage fish spawning across the Salish Sea it is likely not appropriate to use one standard regression and instead field sampling should be conducted to define the relationship. To do this, duplicate sampling using both methods should be done for at least one season along long term monitoring sites to allow the calculation of a corrective factor to equilibrate data for interannual comparison.

Whichever method is chosen, forage fish spawn is a critical life history element for forage fish management that has been largely overlooked outside of Washington State, despite being a cornerstone of functioning marine ecosystems vulnerable to development and water quality degradation. Forage fish spawn sampling should be a high priority for all regions where forage species are present. Either of these methods is appropriate for this work. The most important element is that these assessments are done, and forage fish spawn, and the habitat that supports spawning, be considered a critical resource for conservation management.

Global challenges to our marine system to continue to be grow. Forage fish provide a critical link within our marine systems, and their spawning habitats are therefore critical to conserve. Mapping forage fish egg distribution on intertidal beaches is one effective tool for identifying critical habitat necessary for spawning. Both winnowing methods developed and used by WDFW over the last four decades are valid tools to detect spawning and either may be used to identify intertidal forage fish spawning habitats. As new methods are developed and monitoring techniques are changed it is important extremely important to define clear and strong relationships between historic and new assessment sampling techniques so that invaluable historic data may be used. Such linkages provide an important long term dimension to our management of these important systems as well as a continued broadening of our understanding of forage fish life history, habitat needs, ecosystem function, conservation and restoration.

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Tables

Table 1. Summary of sampling sites, dates, and distances for 2017 winnowing method comparison study

Site	Dates sampled	Linear distance of beach sampled each date (m)
Cline Spit	May 24, June 15	90
Moore	June 12	150
East Elwha Delta	June 12, July 22	2040
West Elwha Delta	June 12	450
Freshwater Bay (FWB)	May 25, June 12	1320
Twins	May 24, June 15	1144

Table 2. **Number of samples by month site for each winnowing method**

Month	Site	Method	Samples	Total Eggs	Mean eggs per sample
May	Cline Spit	Original	2	140	70
		Vortex	2	225	112.5
	Twins	Original	5	200	40
		Vortex	5	254	50.8
	Freshwater Bay	Original	8	0	0
		Vortex	8	2	0.25
June	Cline Spit	Original	2	393	196.5
		Vortex	2	470	235
	Twins	Original	10	1	0.1
		Vortex	10	1	0.1
	Freshwater Bay	Original	8	0	0
		Vortex	8	8	1
	East Delta	Original	16	2	0.125
		Vortex	16	0	0
	Moore	Original	4	0	0
		Vortex	4	0	0
	West Delta	Original	8	0	0
		Vortex	8	0	0

Table 3. Total eggs, by stage, and average number of broods for each site and sampling method

Method and site	.5 - 1 coil	late eyed	1.5 coil	1 - 1.5 coil	1 coil	Avg # broods	Total # of eggs	Total dead	% dead
O	143	2	7	24	8	2.1	736	544	0.74
Cline Spit	26			4	3	1.7	533	500	0.94
East Delta						1	2	2	1
FWB							0		
Moore							0		
Twins	117	2	7	20	5	3	201	42	0.21
West Delta							0		
V	88	3	53	32	7	1.9	960	777	0.81
Cline Spit	2		9	6	6	1.5	695	672	0.97
East Delta							0		
FWB	1					1	10	9	0.9
Moore							0		
Twins	85	3	44	26	1	3.3	255	96	0.38
West Delta							0		
Grand Total	231	5	60	56	15	2	1696	1321	0.78

Table 4: Results of the Kruskal-Wallis test for number of eggs collected with two different methods. The full model contains data from all six sites. The reduced model contains data from Cline Spit and Twins only, which contained fewer zeros than the other sites.

Model	Variable	Chi-Squared	df	p-value
Full	Method	0.82	1	0.37
	Month	12.18	1	0.00048
	Site	58.95	1	< 0.0001
Reduced	Method	0.12	1	0.72
	Month	3.82	1	0.051
	Site	19.22	1	< 0.0001

Figures

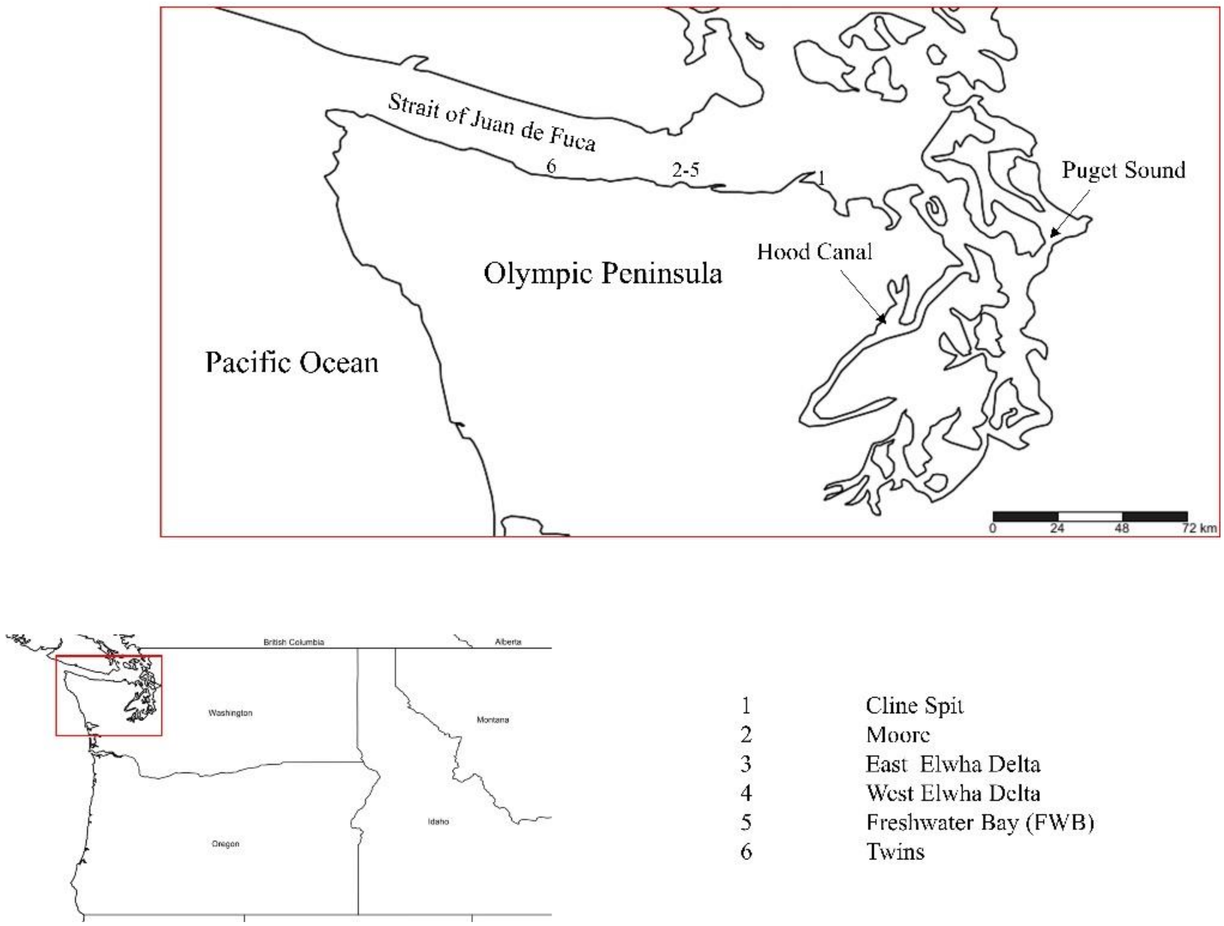

Fig.1. Site map.

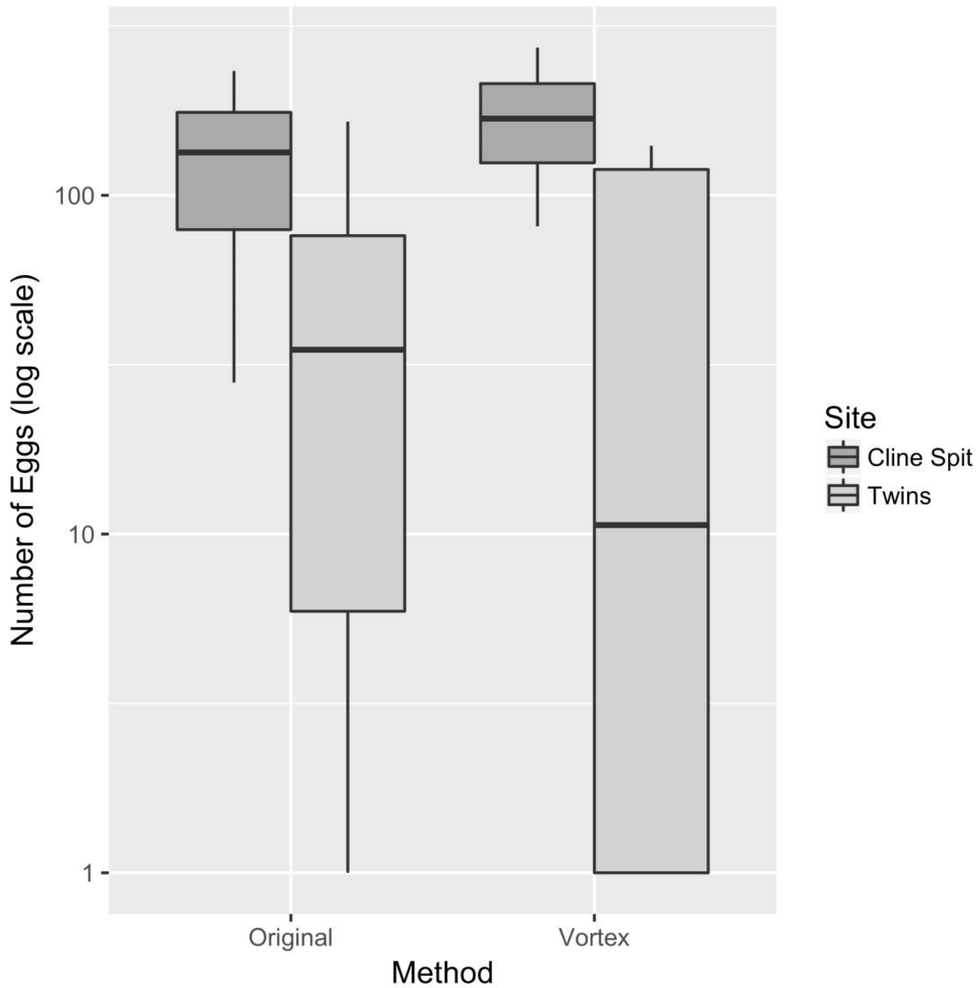

Fig. 2. Box plot of number of eggs collected using two different methods at two sites on the Puget Sound. Note that y-axis is in a log scale.

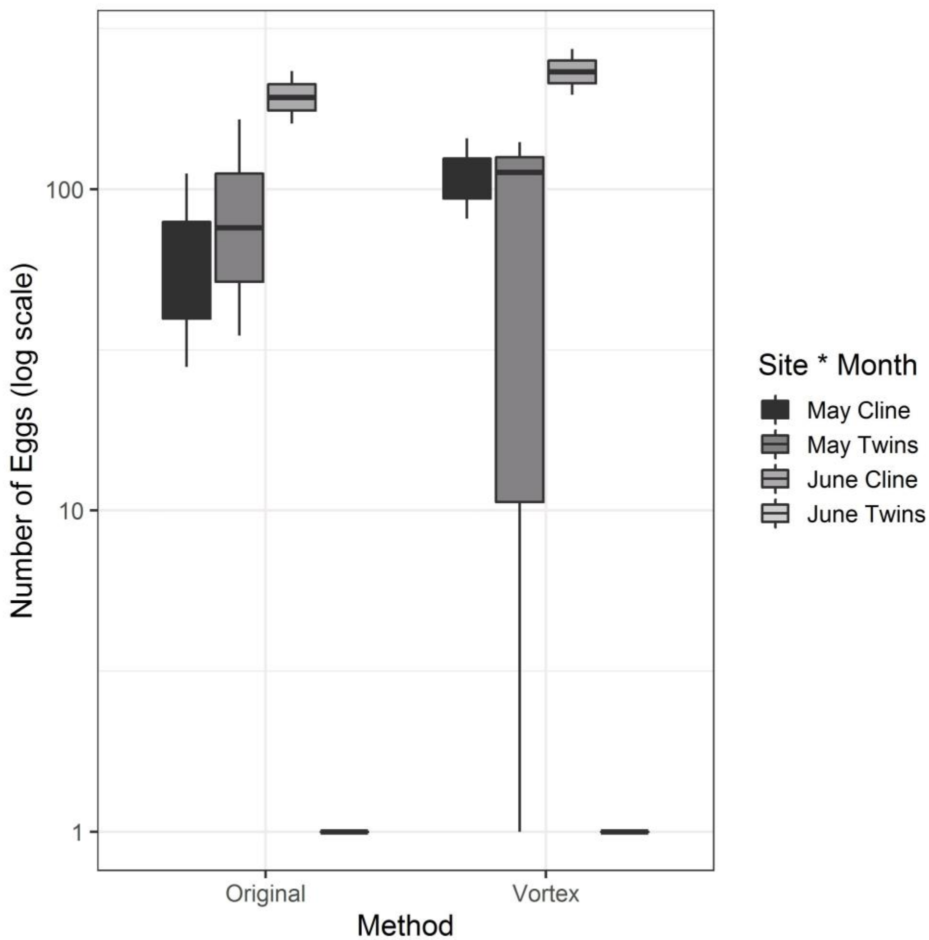

Fig. 3 Box plot of number of eggs collected using two different methods at the two sites on the Puget Sound over two months (May and June). Note that y-axis is in a log scale.

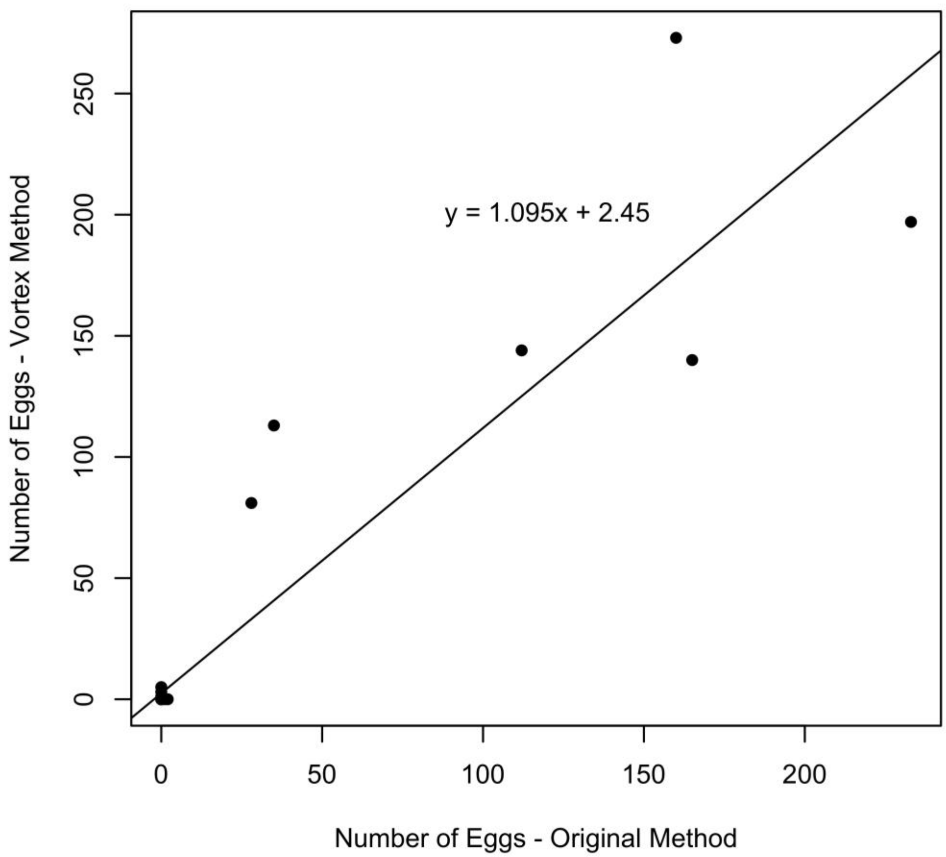

Fig. 4. Linear regression comparing the two different winnowing methods at 6 sampling sites in the Salish Sea. The slope (x=1.095) is not significantly different from x=1 ($p > 0.05$).

Appendix A.

Coastal Watershed Institute Pictures of ‘Vortex’(1-2) and Original’ (3) methods