Comparing the 3rd Generation Environmental Sample Processor to Traditional Laboratory Methodologies for Use in environmental DNA Studies

Edward Good, University of Exeter

Mentors: Kevan Yamahara, Chris Preston, Jim Birch

Summer 2018

Keywords: Carmel River, eDNA, Rainbow trout, Water sampling

ABSTRACT

Due to its fast, cost effective and highly sensitive nature, using environmental DNA (eDNA) as a sampling method has rapidly gained prominence in the surveying of aquatic systems. With such rapid implementation, development and standardization of methodologies is required to ensure comparability between studies. This paper compares traditional laboratory filtration methodologies to filtration using the 3rd Generation Environmental Sample Processor (3G-ESP) by assessing quantities of eDNA from three species from the Carmel River; the Rainbow trout (*Oncorhynchus mykiss*), Striped bass (*Morone saxatilis*), and the New Zealand Mud Snail (NZMS, *Potamopyrgus antipodarum*). Our findings suggest that the 3G-ESP is comparable to traditional peristaltic methods, capturing similar quantities of eDNA for the species. However, the ease of clogging for the 3G-ESP could cause potential issues when comparing eDNA quantities between sample sites. We found eDNA of the Rainbow trout beyond the site of the historic San Clemente dam, where both the Striped bass and the NZMS were absent. Further ecological conclusions will need accurate characterization of the river's abiotic features.

INTRODUCTION

Detecting environmental DNA (eDNA) is a powerful emerging tool for the surveying of aquatic species (Goldberg et al. 2015). Aquatic eDNA primarily consists of organic material such as mucus, feces, urine, skin cells, blood, sperm and eggs, that have been shed or excreted from an organism and remain detectable in the water system (Balsingham et al. 2017). By extracting eDNA from a water sample, an ecosystem can be surveyed in a non-invasive manner, with no impact on the target species (Beja-Pereira et al. 2009; Goldberg et al. 2016). Additionally, eDNA surveys have been shown to be more sensitive than traditional surveying techniques, such as trapping, when the target species occurs in low densities (Thomsen et al. 2012; Dougherty et al. 2016; Valentini et al. 2016). This has allowed eDNA to be particularly successful in assessing the presence of rare and invasive species (Jerde et al. 2010; Tréguier et al. 2014; Xia et al. 2017; Balasingham et al. 2017). eDNA metabarcoding methods, which can screen an entire community's organisms using broad range primers, have also allowed assessments of an aquatic system's biodiversity, with equal or higher sensitivity to traditional methods (Valentini et al. 2016).

In recent years, with the development of quantitative polymerase chain reaction (qPCR), efforts have been made to assess whether the quantity of eDNA found in a system is indicative of the target species' population size (Bohmann et al. 2014). So far, attempts to quantify populations based on eDNA quantities have been hindered by the unknown parameters of residency time, degradation rates and other environmental factors on the detectability of eDNA (Goldberg et al. 2016). Currently, the literature presents highly variable estimates of eDNA residency, ranging from hours over short distances, to up to 12 km (Tillotson et al. 2018; Deiner & Altermatt, 2014). Factors such as mechanical forces, microbial activities, living to dead ratio, and water temperature all influence eDNA's persistence in the environment (Goldberg et al. 2016; Tillotson et al. 2018). Despite these potential influences on eDNA persistence, there are positive correlations between biomass and eDNA concentrations, and in some cases, relative eDNA

concentration does match historical abundance data (Hänfling et al. 2016; Takahara et al. 2012; Pilliod et al. 2013).

This paper, therefore, investigates whether the relative quantities of eDNA from three ecologically linked species, the Rainbow trout (*Oncorhynchus mykiss*), Striped bass (*Morone saxatilis*) and the New Zealand Mud Snail (NZMS, *Potamopyrgus antipodarim*), can give an indication of their abundance and interactions along the Carmel River, California. Rainbow trout, a Pacific salmonid native to California, are listed as threatened or endangered across their natural range, with populations seeing widespread declines in numbers (Ruckelshaus et al. 2002; Gustafson et al. 2007). Anthropogenic threats, such as river dams, have reduced viable trout habitats by as much as 90% (Press et al. 2008). The San Clemente Dam on the Carmel River completely blocked upstream passage of Rainbow trout juveniles, reduced upstream movements of adult Rainbow trout and reduced the survival of smolts moving downstream (Boughton et al. 2016). This dam was removed in 2015, however, it is believed to have had serious negative impacts on the resilience of this local population (Boughton et al. 2016). Other stressors, including avian predation and low river flow from reduced rainfall, can further exacerbate Rainbow trout declines (Osterback et al. 2013; Boughton, 2017).

Invasive species have also contributed to the declines in trout populations, with the Striped bass and the NZMS posing significant threats to Rainbow trout populations. The presence of Striped bass has been predicted to cause a 28% chance of extinction within 50 years for Chinook salmon (*O. tshawytscha*) due to predation on juvenile salmon (Lindley & Mohr, 2003). This interaction is likely to be similar with Rainbow trout, with Striped bass having been observed in the Carmel River. The NZMS can occur in population densities exceeding 100,000 individuals per m^2 outside of their native range, can sequester stream primary production, limit nutrient cycling and become the dominant invertebrate secondary producers by outcompeting native species (Vinson & Baker, 2008; Goldberg et al. 2013). This ecosystem dominance is exacerbated by the NZMSs' indigestibility, with 53.8% of NZMSs passing through Rainbow trout digestive systems alive, causing a 0.14-0.48% reduction in body weight per day (Vinson & Baker, 2008). As Rainbow trout typically consume prey in accordance with their abundance in the

environment, the indigestibility of the NZMS could have significant impacts on Rainbow trout population (Vinson & Baker, 2008).

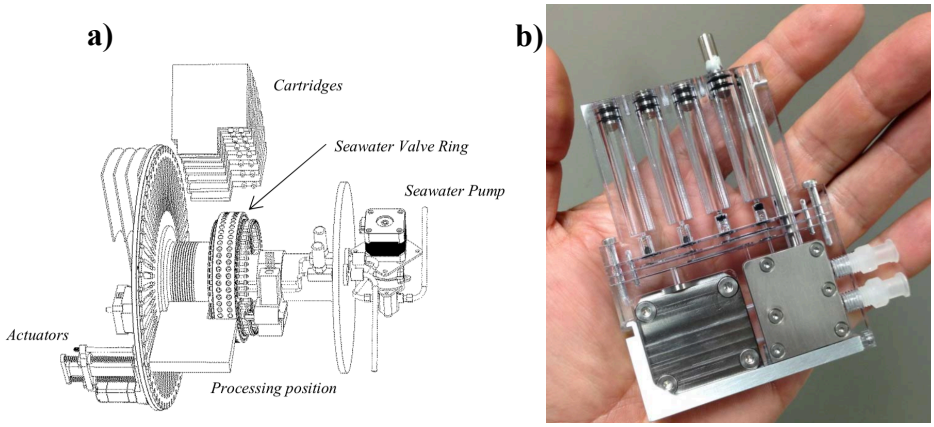

Figure 1. a) exploded diagram of 3G-ESP instrument b) close up image of a single cartridge. Adapted from Pargett et al. 2015.

Additionally, this project also examines the equivalence of two sampling methodologies. The first uses traditional laboratory methods whereas the second uses the 3rd Generation Environmental Sample Processor (3G-ESP) to sample freshwater systems, (Pargett et al. 2015). With the rapid expansion in eDNA sampling, inconsistencies between sampling protocols limit their comparability, and human error inevitably leads to increased risk of false positive detections (Goldberg et al. 2016). Development of products such as the portable ANDe™ system that allows high-throughput eDNA sampling with minimal opportunities for cross contamination, are doing much to standardize the eDNA sampling process (Thomas et al. 2018). The 3G-ESP builds upon this, offering greater deployment versatility; it can be housed within a long-range autonomous underwater vehicle (LRAUV), and, with 60 cartridges housing filters, the 3G-ESP has the capacity for long term, in situ eDNA sample processing (Fig. 1, *adapted from* Pargett et al. 2015). While the 3G-ESP has demonstrated its capabilities in marine systems, it has not been tested in freshwater systems, where factors such as turbidity and sedimentation could impact its effectiveness.

This project has two distinct aims. Firstly, understanding whether the 3G-ESP provides similar results as the current 'gold standard' bench-top filtration systems. This will be the first step in a long-term goal of being able to develop a modular DNA extraction

device to add to the 3G-ESP, allowing complete sample processing in a closed loop system. Secondly, based on the quantities of DNA from the sample sites, we hope to assess whether the concentrations are reflective of species abundance, and thus determine ecological interactions between the species. For example, where exclusionary interactions between Rainbow trout and both the Striped bass and the NZMS occur, we would expect to see an inverse relationship between their respective eDNA concentrations.

MATERIALS AND METHODS

Sampling

Water samples were collected from six sites representing 25 miles of the Carmel River during June 2018 (Fig. 2). Four more samples were collected from the Monterey Bay Aquarium's (MBA) Coastal Stream exhibit that houses Rainbow trout. Water from the exhibit was taken from the municipal water supply, the inflow supply into the tank, within the tank, and from the outflow exiting the tank. At each site, 10 L of water was collected and immediately put on ice to minimize DNA degradation until the sample was filtered.

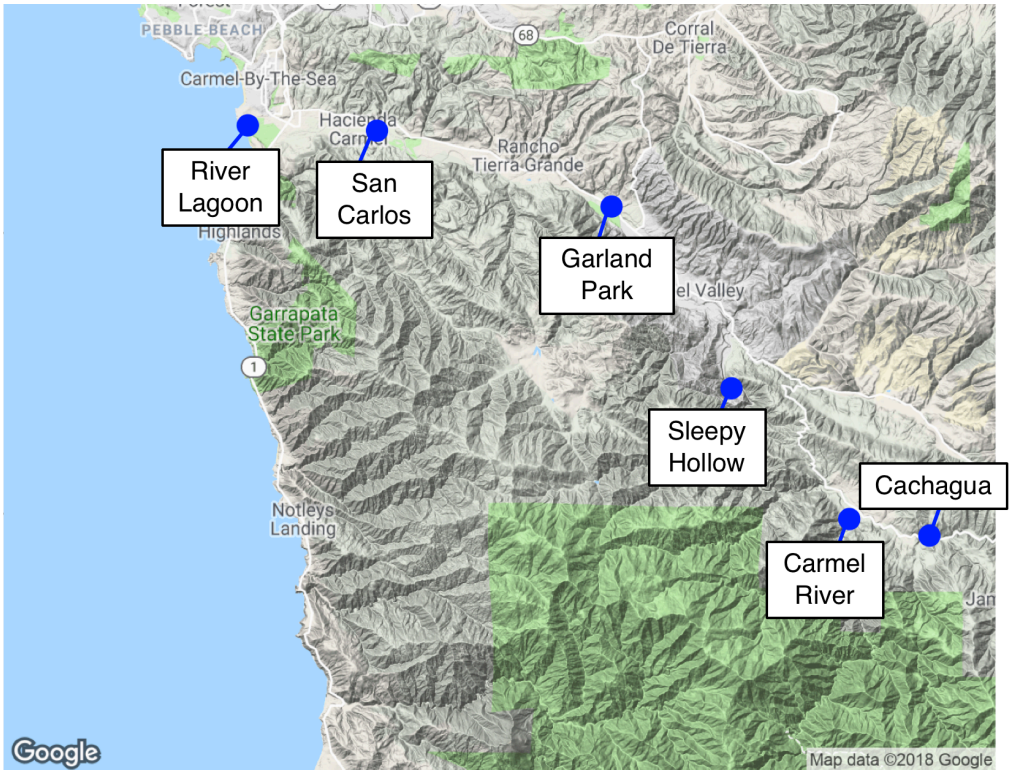

Figure 2. sample sites along the Carmel River. Water was collected over two days (12^{th}-14^{th} June 2018).

Filtration and Extraction

Water samples were filtered within 12 hours of collection. Six 1 L aliquots of the original 10 L were separated out, of which three 1 L aliquots were filtered using traditional laboratory methods, and the other three 1 L aliquots were filtered using the 3G-ESP. This gives each sample site three replicates for both filtration methods. All filters were 25 mm, 0.22 μm Durapore Membrane Filters (Millipore, Temecula, CA, USA).

Traditional laboratory methods used a peristaltic pump (Masterflex L/S Variable-Speed Drive with a Masterflex L/S Easy-Load II Head) to push the water samples through the Swinnex filter holders at 50 mL per minute (Church et al. 2005). Immediately after filtration, filters were removed from the Swinnex housing, rolled into a 2 mL screw cap tube containing 0.25 g of 0.5mm and 0.25 g of 0.1mm glass beads and frozen at -80°C.

For the 3G-ESP, water samples were filtered and preserved using custom 3G-ESP "archive" cartridges (Pargett et al. 2015). Water was pumped through cartridges utilizing a custom ceramic rotating piston pump, where, after filtration, material collected on the filters was preserved utilizing RNAlater (Pargett et al. 2015). The immersed filter was then incubated for 15 min. After the incubation, the RNAlater was expelled from the filter, and the filter was recovered from the cartridge, placed into a 2 mL screw cap tube containing 0.25 g of 0.5mm and 0.25 g of 0.1mm glass beads and frozen at -80°C.

In cases where the 3G-ESP failed to filter the entire 1 L sample, efforts were made to match the volume filtered in the traditional laboratory methods. In two cases (River Lagoon and San Carlos), additional water was filtered using the traditional laboratory technique to provide a comparison between partial filtration and filtering the full liter (Appendix 1).

DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit, with the manufacturer's protocol being modified to include an initial bead-beating step in 900 μl ATL Buffer (Qiagen) (Djurhuus et al. 2017). Total DNA was eluted from filters in 100 uL PCR grade water and aliquoted into 4 – 25 uL volumes and stored at -80°C.

Amplification

Quantification of eDNA was achieved using qPCR with a StepOnePlus™ Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Specific primer sequences for each species tested can be seen in Table 1. Each 20µl reaction contained 1X Environmental Mastermix (Applied Biosystems, Foster City, CA, USA), forward and reverse primers and probe (Table 1) and 2 µl of lysate that contained the template DNA. All samples were run in triplicate. We tested for inhibition by aliquoting a 1:5 dilution, which was run simultaneously with the full concentration DNA solution. PCR conditions had an initial single thermal cycle to 95°C for 10 minutes, followed by 45 cycles of 15 seconds at 95°C and 60 seconds at 60°C. Quantification of samples was determined from standard curves from 6 ten-fold serial dilutions of gBlock® Gene Fragments (Integrated DNA Technologies, Skokie, IL, USA). Replicate standard curves from each qPCR plate were compiled to create a composite standard to ensure all copies were quantified to the same degree. Due to the low efficiency of the NZMS assay (<70%) results were not quantified, instead amplification fluorescence signal was used to assess presence/absence of the intended target gene. All statistical analyses were completed using the statistical software R (v3.5.1).

Table 1. Species specific sequences used in qPCR assays

Species	Gene	Oligonucleotide	Sequence	Concentration (nM)	Amplicon Length (bp)	Reference
O. mykiss	NADH	Forward	5'-AGTCTCTCCCTGTATATCGTC-3'	300	102	Wilcox et al. 2015
		Reverse	5'-GATTTAGTTCATGAAGTTGCGTGAGTA-3'	600		
		Probe	5'-CCAACAACTCTTTAACCATC-3'	250		
M. saxatilis	COI	Forward	5'-TCCCCGAATGAACAACATAAGTT-3'	400	63	Brandl et al. 2015
		Reverse	5'-GAAGCTAGAAGGAGGAGGAAGGA -3'	400		
		Probe	5'-TTGACTGCTTCCCCC-3'	200		
P. antipodarum	cytb	Forward	5'-TGTTTCAAGTGTGCTGGTTTAYA-3'	400	79	Goldberg et al. 2013
		Reverse	5'-CAAATGGRGCTAGTTGATTCTTT-3'	400		
		Probe	5'-CCTCGACCAATATGTAAAT3'	250		

RESULTS

We detected eDNA of Rainbow trout at all sample sites along the Carmel River as well as from the MBA samples, except the municipal water supply. Striped bass was found at all river sites except for the Nason and Cachagua sites, and NZMS was only detected at San Carlos and Garland Park (Fig. 3). All three species were undetected in all control negative samples. For Carmel River samples, mean copy number per mL ranged from 15.7 to 598 for *O. mykiss*, whereas for *M. saxatilis*, mean copy number was much lower, ranging from 1.25 to 3.12 copies per mL (Fig. 3). For the ESP, the mean copy number per mL ranged from 1.25 up to 245, whereas traditional methodologies ranged from 1.43 to 598. *M. saxatilis* had copy numbers lower than our smallest quantity DNA standard, so the conclusions we could draw were limited. While we used copy number of *M. saxatilis* to compare 3G-ESP collection methods with traditional laboratory methods, for ecological comparisons we used gene copy number to simply describe presence/absence.

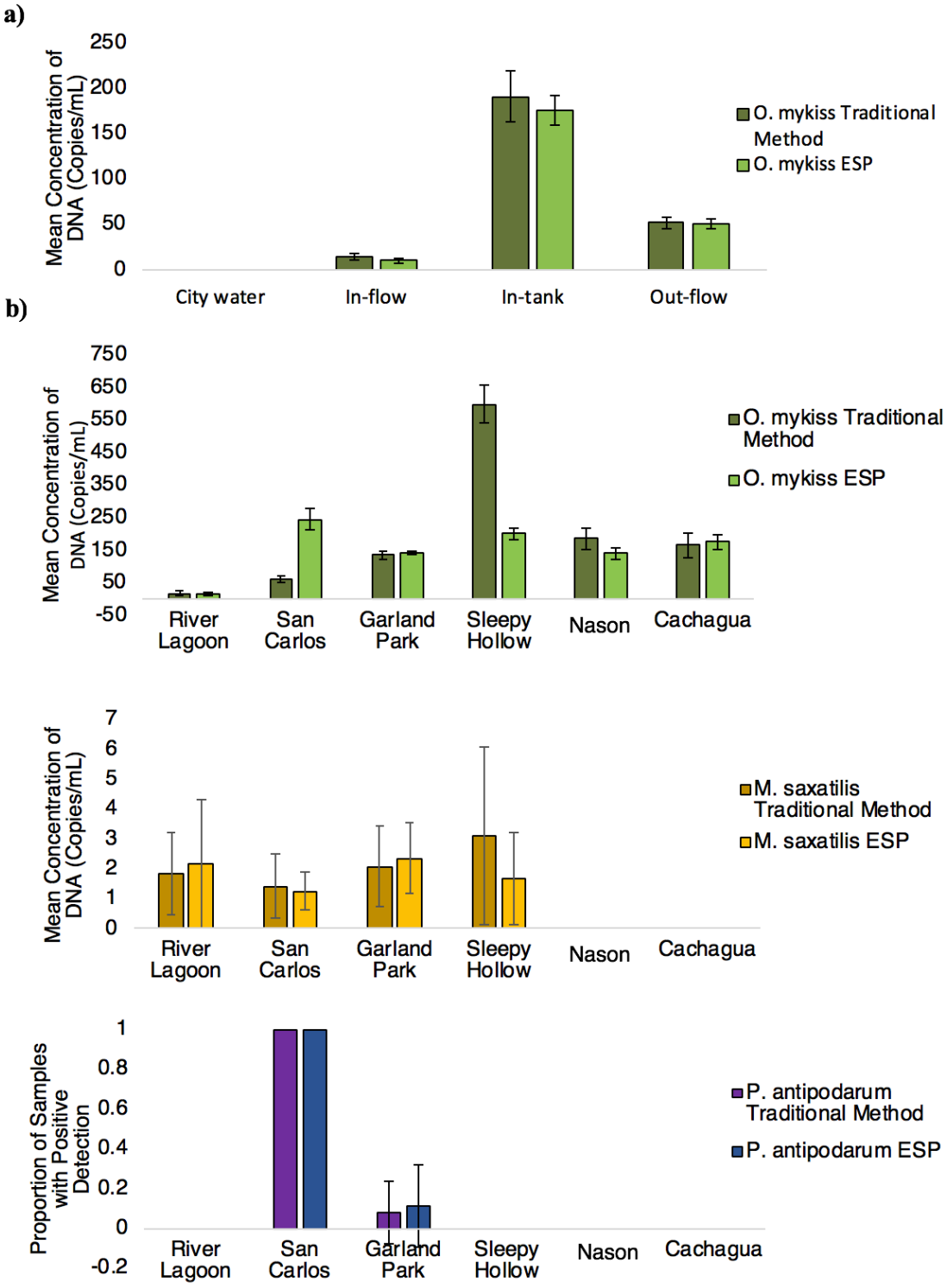

Figure 3. Comparison of 3G-ESP and traditional laboratory methods. a) Monterey Bay Aquarium Coastal Exhibit sample sites, b) Carmel River sample sites. All bars plotted with 95% confidence interval values.

Data was not normally distributed and could not be transformed to achieve normality. For *P. antipodarum*, chi-squared was used on the number of positive/negative detections. No significant relationship was found, indicating that we can accept the null hypothesis that they are from the same sample (Fig. 3). For comparing copy numbers of *O. mykiss* and *M. saxatilis*, data was ranked and tested using an analysis of co-variance, using sample site as the co-variate. For both *O. mykiss* and *M. saxatilis*, the copy numbers detected were found to be significantly the same between the traditional methodology and the 3G-ESP ($F_{1,66}$=130, p=<0.001; $F_{1,63}$=137, p=<0.001; Fig. 4).

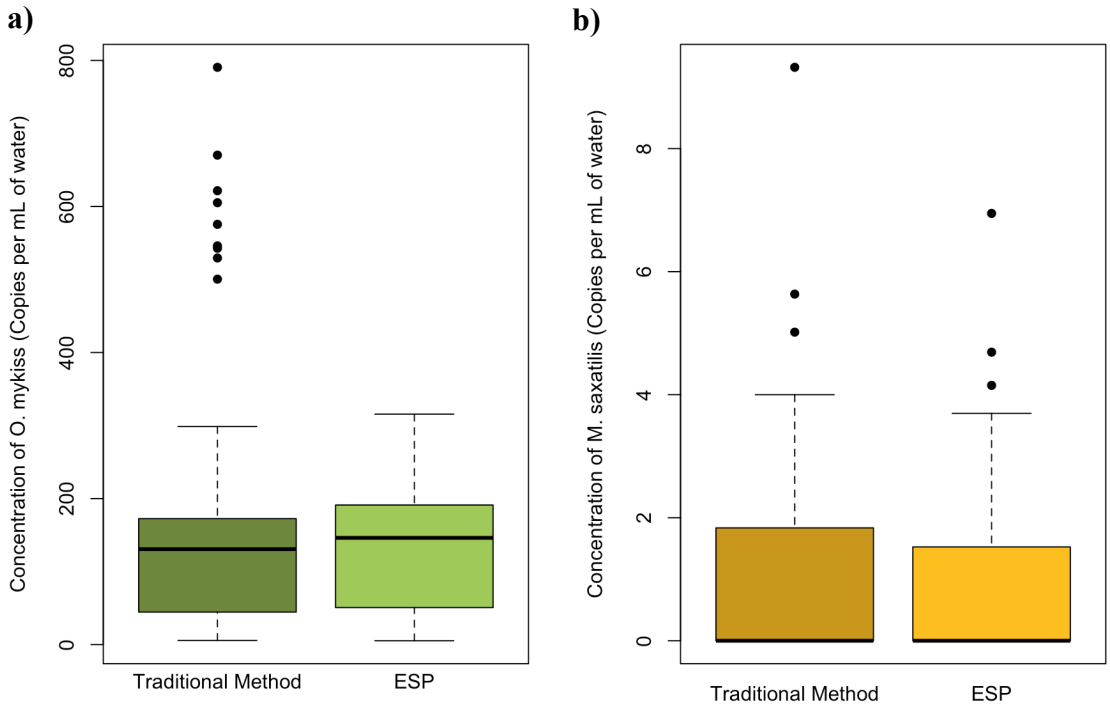

Figure 4. a) *O. mykiss* copy number distribution for both traditional and ESP methods, b) *M. saxatilis* copy number distribution for both traditional and ESP methods. Note the difference in scale between species.

Due to clogging of the filter in the 3G-ESP at low sample volumes, additional filtering was completed using the traditional methodology to determine whether the DNA captured at lower sample volumes is proportional to the amount captured when the full 1 L is filtered. There were significant differences found between partial sample filtering and complete filtering; at River Lagoon, the 1 L sample filter returned significantly fewer DNA copies per mL than both the traditional matched filter amount and the 3G-ESP filter amount using a one-way ANOVA ($F_{2,24}$=12.8, p=<0.001), and at San Carlos, the 1 L

sample filter captured significantly fewer copies per mL than the 3G-ESP ($F_{2,24}$=115, p=<0.001; Fig 5).

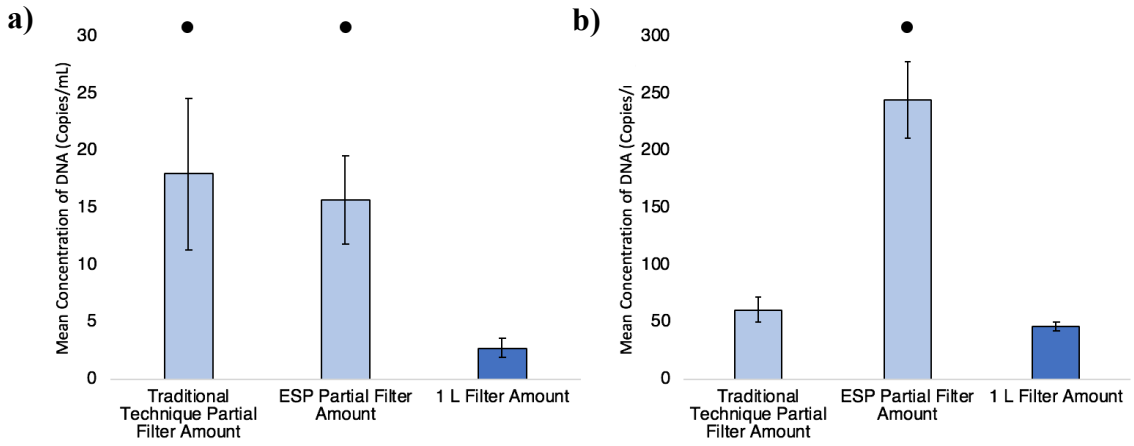

Figure 5. Mean copy numbers of *O. mykiss* captured based on quantity of water filtered. a) River Lagoon site; mean traditional partial filter amount = 400 mL, mean ESP partial filter amount = 377 mL. b) San Carlos site; mean traditional partial filter amount = 383 mL, mean ESP partial filter amount = 377 mL. Dots above bars indicate significant difference to the 1 L filter amount. Bars indicate 95% confidence interval for traditional lab methods.

To see the relationship between ESP-collected samples and traditionally-collected samples, we use linear regression of mean copy number for each methodology at each sample site. There was no significant relationship between the copies per mL captured using the 3G-ESP and the traditional laboratory methods (R^2=0.28, d.f.=7, p=0.14; Fig. 6a). However, due to Sleepy Hollow failing to have matched filter volumes, with the traditional laboratory method filtering a mean of 963 mL and the 3G-ESP filtering a mean of 264 mL, we omitted that data point. With this omission, the regression remained non-significant, but had a higher correlation (R^2=0.39, d.f=6, p=0.096; Fig 6b).

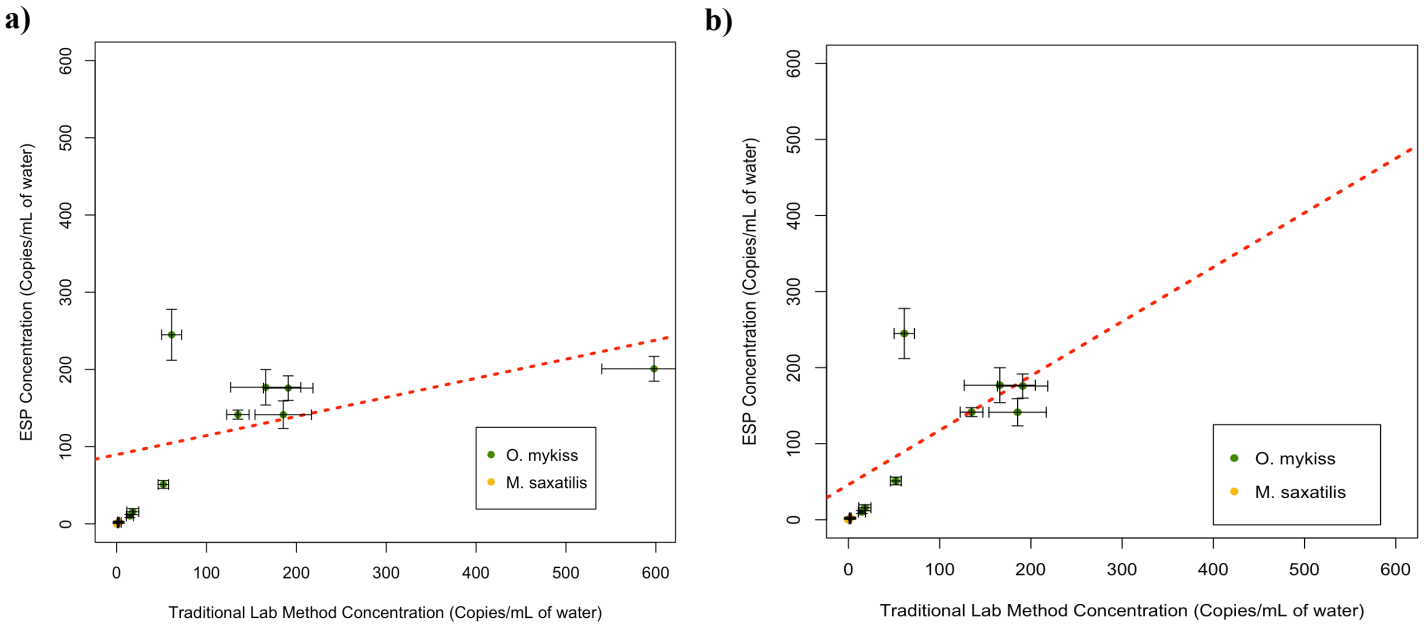

Figure 6. Mean copy numbers of both *O. mykiss* and *M. saxatilis* recorded at each sample site. a) all site locations included. b) Sleepy Hollow site omitted. Horizontal bars indicate 95% confidence interval for traditional lab methods, vertical bars indicate 95% confidence interval for the 3G-ESP. Red dotted line indicates the line of best fit.

To assess the relative variability of the different methodologies, the coefficient of variance was calculated. No significant difference was found between the coefficient of variance using a Wilcoxon rank sum test (Fig. 7).

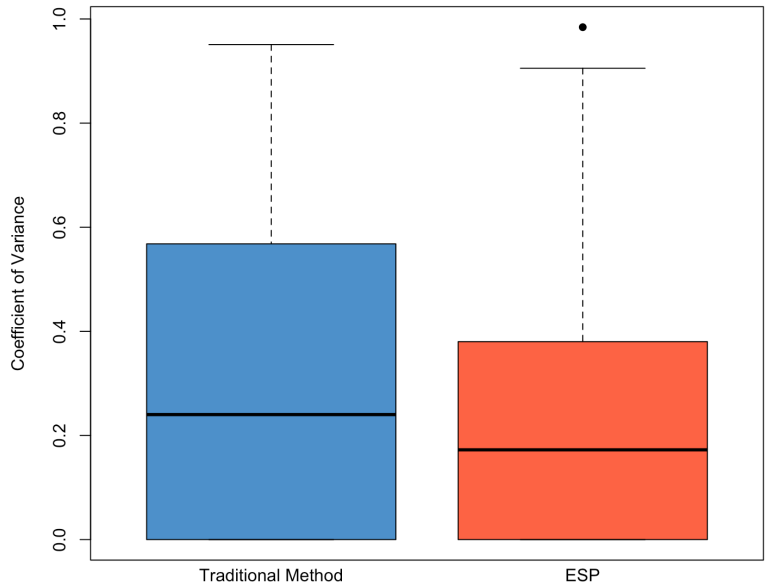

Figure 7. Coefficient of variance for both the traditional laboratory methodology and the 3G-ESP.

Ecological Interaction Results

For ecological results, data from both the 3G-ESP and the traditional laboratory methods were pooled and analyzed together. Due to the impact sample filtration volume had on DNA copies per mL, for the *O. mykiss* results at sites River Lagoon, San Carlos and Sleepy Hollow, only results where the full 1 L sample was filtered were used. There were differing patterns of spatial distribution along the Carmel River based on the species, with only *O. mykiss* being found beyond the historic site of the San Clemente dam. *M. saxatilis* was found present at a high proportion of sample sites up to the San Clemente dam site, whereas *P. antipodarum* was only found consistently at San Carlos, intermittently at Garland Park (2/21 replicates for the site returned a positive identification for the species) and was absent from all other sites (Fig. 8).

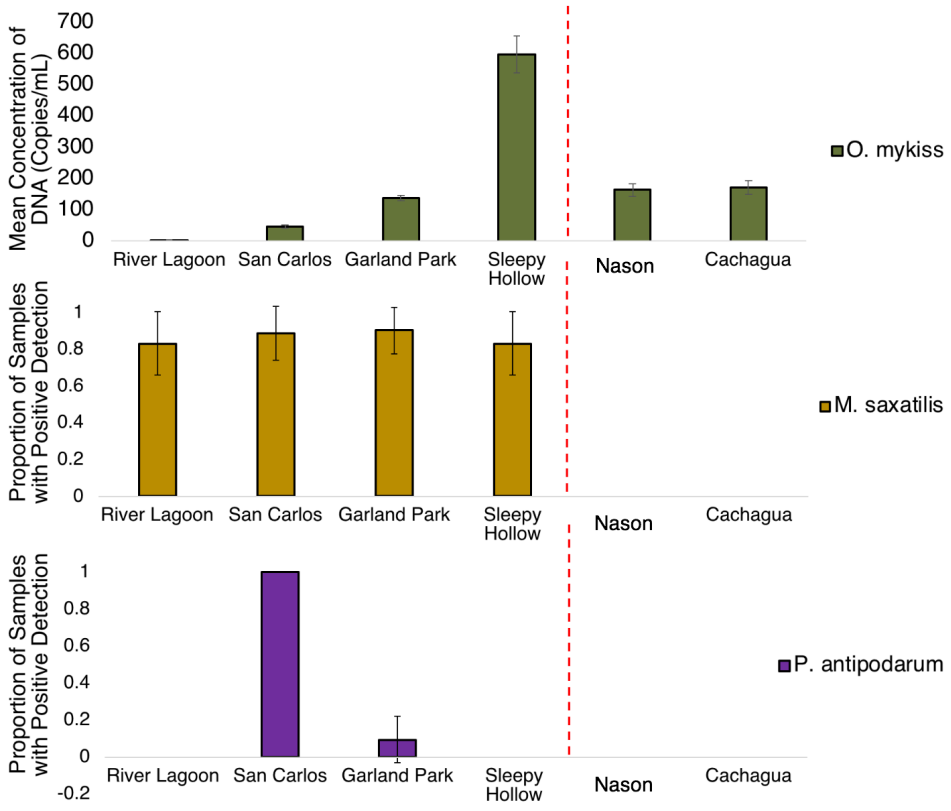

Figure 8. Distribution of species along the Carmel River. Sites ordered according to their river mile, with River Lagoon being closest to the river mouth. Red dashed line indicates historic location of the San Clemente dam.

eDNA concentration for *O. mykiss* was not significantly correlated with location along the river (Spearman's rank correlation, r_s=0.83, n=6, S=6, p=0.058; Fig. 9).

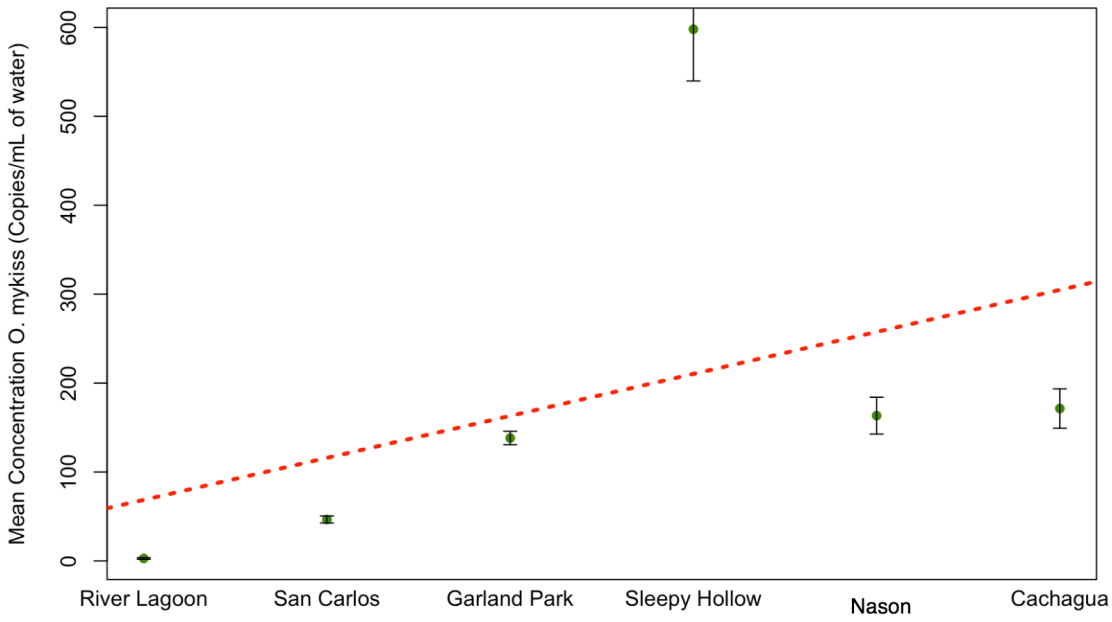

Figure 9. Concentration of *O. mykiss* eDNA concentration, sites ordered according to their river mile, with River Lagoon being closest to the river mouth. Bars indicate 95% confidence interval. (Spearman's rank correlation, r_s=0.83, n=6, S=6, p=0.058).

DISCUSSION

The 3G-ESP is a comparable filtration method to traditional laboratory methodologies in the context of freshwater systems. Both methods detected the same species at the same sites, with similar total mean copies per mL. The ANCOVA, when accounting for site, indicated that both methodologies returned equivalent copy numbers for both Rainbow trout and the Striped bass. Furthermore, there was no difference between the detection rate of the NZMS when comparing 3G-ESP and traditional techniques. Both methods also displayed similar levels of variance. While the correlation between copy numbers was found to be non-significant, even with the outlier removed, a positive relationship is possibly suggested. This relationship may become more apparent with the addition of

more data points; however, this needs further investigation. Regardless, across all other tests the 3G-ESP matches the traditional techniques.

However, where the 3G-ESP falls short of traditional laboratory methods is the ease at which the system clogs, preventing the full filtration of the 1 L sample. While the peristaltic pump was able to force through the full 1 L for every sample site, the 3G-ESP was not able to. As our results show, failing to filter the full liter can drastically alter the quantification results, suggesting that the DNA is not evenly distributed throughout the sample; if all the DNA is captured in the first 300 mL of the sample, and those results are extrapolated out to be compared to sites that have filtered 1 L, then a site with partial sample filtration will appear to have a much higher concentration of DNA copies than had it had the full 1 L filtered. Previous studies have found that eDNA settles very quickly in aquatic systems, with carp eDNA being most commonly found in particle sizes of 1-10 μm, and rarely found suspended in the water column (Turner et al. 2014; Tillotson et al. 2018). Therefore, eDNA is likely to be heterogeneously distributed throughout the samples. Turner et al. (2014) developed isoclines to indicate how much water was needed to be filtered, at a certain filter size, to capture similar quantities of carp eDNA to counteract their rate of filter clogging. For survey sites where filters clogged easily, they recommended choosing a filter with larger pores and filtering a greater sample volume to compensate (Turner et al. 2018). However, whether these isoclines translate to different species or water systems requires further investigation, and would be a valuable tool in standardizing eDNA sample processing.

The distribution and quantity of the eDNA concentrations has given some insights into characterizing the Carmel River. The presence of Rainbow trout DNA beyond the historical site of the San Clemente dam, at similar quantities to sites before the dam, indicates that the removal of the dam has restored the connectivity that was lost during the dam's existence (Boughton et al. 2016). However, the absence of Striped bass beyond the dam site perhaps indicates there may be a barrier to their migration up the river. While Rainbow trout's ability to pass the dam was reduced, they did have access to the river beyond the dam via a fish ladder, while historically Striped bass had no access (Boughton et al. 2016). This may potentially indicate a lag between the dam's removal

and the Striped bass' migration into newly available river. Comparatively, the NZMS's distribution along the river remains constrained to San Carlos and Garland Park. As the NZMS is an aggressively invasive freshwater snail, with few effective predators outside of their natural range, it is unlikely that their distribution is confined due to biotic or abiotic factors (Bersine et al. 2008). Alternatively, their presence in the Carmel River could be recent, and their low detection rate at Garland Park could indicate an expansion of their range up stream.

Regarding the interactions between species, there seemed to be no competitive exclusion between any of the species at any of the sites. While analysis was limited due to the low copy numbers of the Striped bass and the inability to quantify the NZMS assay, no clear pattern emerged from the presence or absence of a species. Beyond the dam site, where Rainbow trout had no competition from the invasive species, there was no significant spike in eDNA concentration. Conversely, there was no reduction in Rainbow trout eDNA concentration at San Carlos, where both NZMS and Striped bass were consistently present.

However, the conclusions that can be drawn about the relative abundance of Rainbow trout, Striped bass, and NZMS populations from this data is restricted by poor characterization of abiotic factors. As eDNA concentration is correlated with biomass (Takahara et al. 2012), and as Striped bass copy numbers were consistently lower than Rainbow trout copy numbers, it is likely that the Rainbow trout population is larger than the bass population. This is supported by the numbers of fish seen in recent conservation efforts; 3,000 Rainbow trout were collected via electroshock along the Carmel River in June 2018, whereas, anecdotally, fewer than 20 Striped bass have been seen. However, the lack of characterization of the abiotic features of the different sample sites severely hinders further analysis. Turbidity, pH, flow rate, living to dead ratio, water volume, sediment type, and exposure to sunlight were all unaccounted for, which can all individually affect the residency of the DNA in the system (Goldberg et al. 2016; Tillotson et al. 2018). Even changes in diet can influence eDNA output (Bohmann et al. 2014). Due to the dynamic nature of river systems, a concentration of eDNA at one site is unlikely to be comparable at another unless these factors are considered (Goldberg et al. 2016). While no correlation was found between sample site and Rainbow trout copy

number, there does appear to be increasing quantity up until the San Clemente dam site, where it then decreases. However, this relationship may simply be reflecting increasing DNA quantity in relation to river flow rather than Rainbow trout population size; anecdotally, while River Lagoon had the smallest quantity of Rainbow trout DNA detected, it was also the largest body of water, exposed to the greatest amount of sunlight, and so the eDNA is more likely to be less concentrated than samples at Cachagua, which had a much more restricted flow. Therefore, any nuance in the relationship between species' eDNA concentration can only be extracted in river systems that are well characterized (Tillotson et al. 2018).

Additionally, this data set is only representative of two days, and may not be representative of a broader pattern within the river system. eDNA degradation, in some stream reaches, has been found to occur over hundreds of meters, with concentrations varying significantly from day to day (Goldberg et al. 2016; Tillotson et al. 2018). Surveying systems over greater temporal scales could reduce some of the noise that occurs from day to day variation, and become more reflective of broader ecological patterns. This is one of the long-term goals of the 3G-ESP; to allow the 3G-ESP to remain in situ within the river system and sample water daily over weeks-to-months. This would have numerous advantages over current sampling methods, which often occur once/month (e.g., USGS, pers. comm.), as its automation would reduce the labor hours, cross contamination risk and increase replicability between samples.

CONCLUSIONS/RECOMMENDATIONS

The 3G-ESP is a viable method of sampling freshwater systems for eDNA when compared to traditional laboratory methods for presence/absence studies. However, the ease at which the 3G-ESP clogs is something that needs to be considered in future. Workarounds, such as larger filter pores while filtering a greater sample volume, require further investigation and would need to be implemented in order for sample sites to be comparable. Ecological conclusions could be drawn from the distribution and respective volumes of the eDNA found in the Carmel River, however, only at the most basic level. Our data suggests that the Rainbow trout population is larger than the Striped bass, and

that Rainbow trout have access to regions beyond the historic San Clemente dam site, whereas the Striped bass and NZMS are more restricted within the river. For higher resolution data, accurate characterization of potential biotic and abiotic factors that could affect the residency or detection of eDNA must also be made before conclusions are drawn. Future studies should focus on which factors have the greatest influence in determining eDNA detection, and which are required to adequately describe the river system.

ACKNOWLEDGEMENTS

Thanks to my mentors, Kevan Yamahara, Chris Preston and Jim Birch for their guidance and assistance throughout this project, as well as the rest of the ESP lab. Thanks to Kris Walz for her advice throughout the lab work. The project would also have been impossible without the aid of Brian LeNeve, of the Carmel River Steelhead Association for facilitating the water sample collections along the Carmel River, and Kasie Regnier, for her assistance in the water sampling of the Monterey Bay Aquarium Coastal Exhibit. Thank you to George Matsumoto for the opportunity to be part of MBARI's internship program and all the opportunities associated with it. Thank you to MBARI, the David and Lucille Packard Foundation, and NOAA (NA15OAR4320064) for providing funds that made this project possible.

References:

Balasingham, K. D., Walter, R. P., Mandrak, N. E., & Heath, D. D. (2018). Environmental DNA detection of rare and invasive fish species in two Great Lakes tributaries. *Molecular Ecology*, *27*(1), 112–127. https://doi.org/10.1111/mec.14395

Beja-Pereira, A., Oliveira, R., Alves, P. C., Schwartz, M. K., & Luikart, G. (2009). Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources*, *9*(5), 1279–1301. https://doi.org/10.1111/j.1755-0998.2009.02699.x

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., … de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution*, *29*(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003

Boughton, D. (2017). *Data Summary – Carmel River steelhead population 2013 - 2016*.

Brandl, S., Schumer, G., Schreier, B. M., Conrad, J. L., May, B., & Baerwald, M. R. (2015). Ten real-time PCR assays for detection of fish predation at the community level in the San Francisco Estuary-Delta. *Molecular Ecology Resources*, *15*(2), 278–284. https://doi.org/10.1111/1755-0998.12305

Church, M. J., Jenkins, B. D., Karl, D. M., & Zehr, J. P. (2005). Vertical distributions of nitrogen-fixing phylotypes at Stn ALOHA in the oligotrophic North Pacific Ocean. *Aquatic Micobial Ecology*, *38*, 3–14.

Dougherty, M. M., Larson, E. R., Renshaw, M. A., Gantz, C. A., Egan, S. P., Erickson, D. M., & Lodge, D. M. (2016). Environmental DNA (eDNA) detects the invasive rusty crayfish Orconectes rusticus at low abundances. *Journal of Applied Ecology*, *53*(3), 722–732. https://doi.org/10.1111/1365-2664.12621

Goldberg, C. S., Sepulveda, A., Ray, A., Baumgardt, J., & Waits, L. P. (2013). Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science*, *32*(3), 792–800. https://doi.org/10.1899/13-046.1

Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2015). Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation*, *183*, 1–3. https://doi.org/10.1016/j.biocon.2014.11.040

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., … Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Gustafson, R. G., Waples, R. S., Myers, J. M., Weitkamp, L. A., Bryant, G. J., Johnson, O. W., & Hard, J. J. (2007). Pacific Salmon Extinctions : Quantifying Lost and Remaining Diversity. *Conservation Biology*, *21*(4), 1009–1020. https://doi.org/10.1111/j.1523-1739.2007.00693.x

Hänfling, B., Handley, L. L., Read, D. S., Hahn, C., Li, J., Nichols, P., … Winfield, I. J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, *25*(13), 3101–3119. https://doi.org/10.1111/mec.13660

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*(2), 150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x

Lindley, S. T., & Michael, M. S. (2003). Modeling the effects of striped bass (*Morone saxatilis*) on the population viability of Sacramento River winter-run chinook salmon (*Oncorhynchus tshawytscha*). *Fishery Bulletin*, *101*, 321-331.

Osterback, A. M. K., Frechette, D. M., Shelton, A. O., Hayes, S. A., Bond, M. H., Shaffer, S. A., & Moore, J. W. (2013). High predation on small populations: Avian predation on imperiled salmonids. *Ecosphere*, *4*(9), 1–21. https://doi.org/10.1890/ES13-00100.1

Pargett, D. M., Birch, J. M., Preston, C. M., Ryan, J. P., Zhang, Y., & Scholin, C. A. (2015). Development of a mobile ecogenomic sensor. *OCEANS 2015 - MTS/IEEE Washington*, (6187530). https://doi.org/10.23919/OCEANS.2015.7404361

Pess, G. R., McHenry, M. L., Beechie, T. J., & Davies, J. (2008). Biological Impacts of the Elwha River Dams and Potential Salmonid Responses to Dam Removal. *Northwest Science*, *82*(sp1), 72–90. https://doi.org/10.3955/0029-344X-82.S.I.72

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., Waits, L. P., & Richardson, J. (2013). Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(8), 1123–1130. https://doi.org/10.1139/cjfas-2013-0047

Ruckelshaus, M. H., Levin, P., Johnson, J. B., & Kareiva, P. M. (2002). The Pacific Salmon Wars: What Science Brings to the Challenge of Recovering Species. *Annual Review of Ecology and Systematics*, *33*(1), 665–706. https://doi.org/10.1146/annurev.ecolsys.33.010802.150504

Takahara, T., Minamoto, T., & Doi, H. (2013). Using Environmental DNA to Estimate the Distribution of an Invasive Fish Species in Ponds. *PLoS ONE*, *8*(2). https://doi.org/10.1371/journal.pone.0056584

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. *PLoS ONE*, *7*(4), 3–10. https://doi.org/10.1371/journal.pone.0035868

Thomas, A. C., Howard, J., Nguyen, P. L., Seimon, T. A., & Goldberg, C. S. (2018). ANDe^{TM}: A fully integrated environmental DNA sampling system. *Methods in Ecology and Evolution*, 1379–1385. https://doi.org/10.1111/2041-210X.12994

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. *PLoS ONE*, *7*(8), 1–9. https://doi.org/10.1371/journal.pone.0041732

Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance

at fine spatial and temporal scales. *Biological Conservation*, *220*(January), 1–11. https://doi.org/10.1016/j.biocon.2018.01.030

Tréguier, A., Paillisson, J. M., Dejean, T., Valentini, A., Schlaepfer, M. A., & Roussel, J. M. (2014). Environmental DNA surveillance for invertebrate species: Advantages and technical limitations to detect invasive crayfish Procambarus clarkii in freshwater ponds. *Journal of Applied Ecology*, *51*(4), 871–879. https://doi.org/10.1111/1365-2664.12262

Turner, C. R., Barnes, M. A., Xu, C. C. Y., Jones, S. E., Christopher, L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution*, (5), 676–684. https://doi.org/10.1111/2041-210X.12206

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. https://doi.org/10.1111/mec.13428

Vinson, M. R., & Baker, M. A. (2008). Poor growth of Rainbow Trout fed NZMS.pdf. *North American Journal of Fisheries Management*, *28*, 701–709.

Wilcox, T. M., Carim, K. J., McKelvey, K. S., Young, M. K., & Schwartz, M. K. (2015). The dual challenges of generality and specificity when developing environmental DNA markers for species and subspecies of Oncorhynchus. *PLoS ONE*, *10*(11), 1–13. https://doi.org/10.1371/journal.pone.0142008

Xia, Z., Zhan, A., Gao, Y., Zhang, L., Haffner, G. D., & MacIsaac, H. J. (2018). Early detection of a highly invasive bivalve based on environmental DNA (eDNA). *Biological Invasions*, *20*(2), 437–447. https://doi.org/10.1007/s10530-017-1545-7

Appendix 1. Breakdown of sample sites, sample quantity filtered, and lysis transfer volume.

KEY
GA = garland park
CA = Cachagua
NA = Nason
SH = Sleepy Hollow
SC = San Carlos
RL = River Lagoon
CW = City Water
IF = In-flow
IT = In-tank
OF = Out-flow

Collection Date	Sample	Bench/ESP	mL filtered	cartridge position	frozen date	extraction date	lysis transfer volume
6/12/18	EXTRA1	Bench	990	n/a	6/12/18	6/15/18	600
6/12/18	EXTRA2	Bench	1050	n/a	6/12/18	6/15/18	600
6/12/18	NEG1	Bench	1000	n/a	6/12/18	6/29/16	600
6/12/18	NEG2	Bench	1000	n/a	6/12/18	6/29/16	600
6/12/18	NEG3	Bench	1000	n/a	6/12/18	6/29/16	600
6/12/18	GP1	Bench	1010	n/a	6/12/18	6/28/16	600
6/12/18	GP2A	Bench	360	n/a	6/12/18	6/28/16	600
6/12/18	GP2B	Bench	740	n/a	6/12/18	6/28/16	600
6/12/18	GP3	Bench	1030	n/a	6/12/18	6/28/16	600
6/12/18	CA1	Bench	980	n/a	6/12/18	6/26/18	600
6/12/18	CA2	Bench	990	n/a	6/12/18	6/26/18	600
6/12/18	CA3	Bench	940	n/a	6/12/18	6/26/18	600
6/12/18	NA1	Bench	880	n/a	6/12/18	6/26/18	600
6/12/18	NA2	Bench	800	n/a	6/12/18	6/26/18	600
6/12/18	NA3	Bench	800	n/a	6/12/18	6/26/18	600
6/12/18	NA END	Bench	930	n/a	6/13/18	6/28/16	600
6/14/18	NEG1	Bench	940	n/a	6/14/18	6/28/16	600
6/14/18	NEG2	Bench	1000	n/a	6/14/18	6/28/16	600
6/14/18	NEG3	Bench	930	n/a	6/14/18	6/28/16	600
6/14/18	SH1	Bench	930	n/a	6/14/18	6/29/16	600
6/14/18	SH2	Bench	930	n/a	6/14/18	6/29/16	600
6/14/18	SH3	Bench	1030	n/a	6/14/18	6/29/16	600

6/14/18	SC1	Bench	380	n/a	6/14/18	6/29/16	600
6/14/18	SC1+	Bench	950	n/a	6/14/18	7/2/18	600
6/14/18	SC2	Bench	390	n/a	6/14/18	6/29/16	600
6/14/18	SC2+	Bench	980	n/a	6/14/18	7/2/18	600
6/14/18	SC3	Bench	380	n/a	6/14/18	7/2/18	600
6/14/18	SC3+	Bench	870	n/a	6/14/18	7/2/18	600
6/14/18	RL1	Bench	390	n/a	6/14/18	6/26/18	600
6/14/18	RL1+	Bench	890	n/a	6/14/18	7/2/18	600
6/14/18	RL2	Bench	410	n/a	6/14/18	6/26/18	600
6/14/18	RL2+	Bench	820	n/a	6/14/18	7/2/18	600
6/14/18	RL3	Bench	400	n/a	6/14/18	7/2/18	600
6/14/18	RL3+	Bench	810	n/a	6/14/18	7/2/18	600
6/14/18	NEG END	Bench	1000	n/a	6/15/28	7/2/18	600
6/19/18	NEG1	Bench	1010	n/a	6/19/18	6/21/18	0
6/19/18	NEG2	Bench	1065	n/a	6/19/18	6/21/18	600
6/19/18	NEG3	Bench	1065	n/a	6/19/18	6/26/18	600
6/19/18	CW1	Bench	990	n/a	6/19/18	6/21/18	600
6/19/18	CW2	Bench	1110	n/a	6/19/18	6/21/18	600
6/19/18	CW3	Bench	1130	n/a	6/19/18	6/21/18	600
6/19/18	IT1	Bench	1040	n/a	6/19/18	6/21/18	600
6/19/18	IT2	Bench	1010	n/a	6/19/18	6/21/18	600
6/19/18	IT3	Bench	1010	n/a	6/19/18	6/21/18	600
6/19/18	IF1	Bench	1005	n/a	6/19/18	6/26/18	600
6/19/18	IF2	Bench	1035	n/a	6/19/18	6/26/18	600
6/19/18	IF3	Bench	990	n/a	6/19/18	6/26/18	600
6/19/18	OF1	Bench	1010	n/a	6/19/18	6/22/18	600
6/19/18	OF2	Bench	1010	n/a	6/19/18	6/22/18	600
6/20/18	OF3	Bench	980	n/a	6/20/18	6/22/18	600
6/12/18	NEG	ESP	1000	60	6/13/28	6/28/16	600
6/12/18	GP1	ESP	988	59	6/13/28	6/28/16	600
6/12/18	GP2	ESP	982	58	6/13/28	6/28/16	600
6/12/18	GP3	ESP	994	57	6/13/28	6/28/16	600
6/12/18	CA1	ESP	884	56	6/13/28	6/28/16	600
6/12/18	CA2	ESP	934	55	6/13/28	6/28/16	300
6/12/18	CA3	ESP	990	54	6/13/28	6/28/16	580
6/12/18	NA1	ESP	608	53	6/13/28	6/28/16	600
6/12/18	NA2	ESP	606	52	6/13/28	6/29/18	600
6/12/18	NA3	ESP	630	50	6/13/28	6/29/18	600
6/12/18	NEG	ESP	988	49	6/13/28	7/2/18	600
6/14/18	NEG	ESP	1000	19	6/14/18	7/2/18	600
6/14/18	SH1	ESP	278	18	6/14/18	6/29/18	600

6/14/18	SH2	ESP	294	17	6/14/18	6/29/18	600
6/14/18	SH3	ESP	221	16	6/14/18	6/29/18	600
6/14/18	SC1	ESP	369	15	6/14/18	6/29/18	600
6/14/18	SC2	ESP	384	14	6/14/18	6/29/18	600
6/14/18	SC3	ESP	377	13	6/14/18	6/29/18	600
6/14/18	RL1	ESP	378	12	6/14/18	7/2/18	600
6/14/18	RL2	ESP	379	11	6/14/18	7/2/18	600
6/14/18	RL3	ESP	374	10	6/14/18	7/2/18	600
6/14/18	NEG END	ESP	1000	9	6/15/18	7/2/18	600
6/19/18	NEG	ESP	1000	42	6/19/18	7/2/18	600
6/19/18	IT1	ESP	1000	41	6/19/18	6/22/18	600
6/19/18	IT2	ESP	1000	40	6/19/18	6/22/18	600
6/19/18	IT3	ESP	1000	39	6/19/18	6/22/18	600
6/19/18	IF1	ESP	1000	38	6/20/18	6/26/18	600
6/19/18	IF2	ESP	1000	37	6/20/18	6/26/18	600
6/19/18	IF3	ESP	1000	36	6/20/18	6/26/18	600
6/19/18	OF1	ESP	1000	35	6/20/18	6/22/18	600
6/19/18	OF2	ESP	1000	34	6/20/18	6/22/18	600
6/19/18	OF3	ESP	1000	33	6/20/18	6/26/18	600
6/20/18	NEG END	ESP	1000	32	6/20/18	7/2/18	600