Spring 2023

The effects of diatom-specific polyunsaturated aldehydes on larval zebrafish (Danio rerio)

Rachel Raymer
Western Washington University, r.rachel.r@gmail.com

The effects of diatom-specific polyunsaturated aldehydes on larval zebrafish (*Danio rerio*)

By

Rachel Raymer

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

ADVISORY COMMITTEE

Dr. Brady Olson, Chair

Dr. James Cooper

Dr. Karin Lemkau

GRADUATE SCHOOL

David L. Patrick, Dean

Master's Thesis

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Rachel Raymer

4/30/2023

The effects of diatom-specific polyunsaturated aldehydes on larval zebrafish (*Danio rerio*)

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
Rachel E. Raymer
May 2023

ABSTRACT

Diatoms are ubiquitous in marine planktonic and benthic environments and are common in diets for many lower-trophic organisms. Certain species of diatoms produce polyunsaturated aldehydes (PUAs) that can exist in particulate and dissolved forms. Diatom PUAs are known for negatively affecting the fecundity of their primary consumers, including invertebrate grazers like copepods and echinoderms. However, little is known about the effects of diatom PUAs on vertebrates that may be exposed to dissolved or ingested PUAs due to overlapping distribution with diatom populations. The purpose of this study was to test whether dissolved diatom PUAs affect the early life stages of a model fish, *Danio rerio* (zebrafish). To test this, zebrafish embryos and larvae were exposed to proportionally increasing mixtures of the dissolved diatom PUAs 2E,4E-decadienal, 2E,4E-octadienal and 2E,4E-heptadienal. Under PUA exposure three metrics of fitness were assessed: embryo heart rate, larval size at hatch, and pre-feeding rate of mortality. In the embryo heart rate experiment, embryos exposed at 24 hours post fertilization (hpf) experienced decreased average heart rate after 2 days of PUA exposure. Embryos 24 hpf exposed to PUA mixtures for 6 days showed a reduction in size in comparison to embryos from controls. Embryos exposed to PUAs from 2 hpf until death showed lower survivorship compared to larvae in controls. The results of this study suggest that larval fish that are sympatric with PUA producing diatoms during their embryonic and larval stages may be susceptible to detrimental effects from PUA exposure.

Acknowledgements

I would like to acknowledge first and foremost my advisor, Dr. Brady Olson, for sticking with me throughout this experience and not letting me give up. I would also like to thank Dr. James Cooper for the endless stream of zebrafish embryos on which to base this research. And last but not least, Dr. Karin Lemkau, for assisting with the chemistry side of this project.

Thank you also to my undergraduate assistant (now a Bachelor of Science!) Soraya Jessa for helping me record and analyze 100+ fish heart videos, and for being willing to jump in and help on any other projects I had going on.

I also can't forget my fellow Olson lab mate (and chemist) Jeremy Johnson, who rescued me from some last-minute concentration recalculations when it seemed like there was no hope.

My thanks, also, to Juan Pablo Carreón Hidalgo at Universidad Autónoma Metropolitana, Iztapalapa. Even though I was a complete stranger hundreds of miles away, he was happy to lend me his R expertise multiple times to help me make the perfect visuals of my nested data.

And, of course, to my friends and family.

Science is conducted by communities, and this thesis would not exist without the support of these wonderful people. Thank you!

Table of Contents

List of Figures

List of Tables

INTRODUCTION

Diatoms are a lineage of microscopic algal phytoplankton that are an important group in marine ecosystems because of their basal trophic position as primary producers and their prominent role in global biogeochemical cycles. Diatoms produce more oxygen than all of the world's rainforests combined, support global fisheries, and contribute to the biological carbon pump by sequestering atmospheric carbon dioxide in their bodies, which sink to depth upon cell death (Field *et al.*, 1998; Smetacek, 1999; Tréguer *et al.*, 2017). They can be especially abundant in coastal waters due to the increased presence of nutrients from terrestrial environments and from seasonal upwelling (Malviya et al., 2016). This high biomass of diatoms in coastal environments means strong competition pressure for resources within diatom communities and from other primary producer functional groups (Zhou et al., 2017).

To gain a competitive advantage for resources, some diatoms produce and emit bioactive chemicals that can have effects across trophic levels (Pohnert, 2005; Franz*é* et al., 2018). These chemicals can lead to reduced herbivore grazing and decreased growth of co-occurring phytoplankton populations (Ribalet et al., 2007a; Romano et al., 1999). Oxylipins, a compound within this array of diatom-produced chemicals, are a product of the lipoxidation of polyunsaturated fatty acids (PUFAs). Oxylipins have displayed high bioactivity in plants, algae, and animals (Barbosa et al., 2016; Prost et al., 2005; Tourdot et al., 2014). Some diatoms, upon damage to the diatom cell wall, intracellularly produce oxylipins called polyunsaturated aldehydes (PUAs), biomolecules composed of a chain lipid with an attached oxygen atom (Caldwell et al., 2009; Fontana et al., 2007). The length of the lipid chain determines the bioactivity of the molecule, with longer chained molecules being associated with increased

bioactive effects on other organisms (Adolph et al., 2003; Ceballos & Ianora, 2003). While these molecules can confer fitness benefits to diatoms, the full extent of each unique PUA's effects and range of biological targets is still being explored (Galasso et al., 2020; Ruocco et al., 2020).

Bioactivity studies on diatom PUAs have historically focused on the invertebrate predators of diatoms, such as pelagic copepods and sea urchin larvae (Ianora et al., 2011; Koski et al., 2008; Lettieri et al., 2015; Ribalet et al., 2007; Ribalet et al., 2014; Romano et al., 2010; Ruocco et al., 2019; Varella et al., 2014; Wolfram et al., 2014). A consistent observation in these studies is that PUAs negatively affect grazer fecundity (Caldwell, 2009; Miralto et al., 1999). In a pioneering study on the effects of PUAs on copepod grazers, three specific diatom PUAs were identified as the compounds responsible for these deleterious effects: 2E,4Z,7Z-decatrienal, 2E,4Z,6Z-decatrienal, and 2E,4E-decadienal (Miralto et al., 1999). This study also highlighted a negative correlation between *in situ* diatom biomass and copepod reproductive success in the Adriatic Sea, suggesting that increased consumption of diatom PUAs negatively affected fecundity.

Since the identification of those three PUAs as a causative agent for reduced grazer fecundity, additional diatom PUAs have been isolated, identified, and explored for negative bioactive effects. Ianora et al. (2010) reviewed the effects of a range of diatoms on copepod fecundity and showed that, of the 21 species tested, all 21 produced negative effects on at least one of four different copepod reproductive criteria (egg production rate, hatching success, morphologically abnormal larvae, and development from naupliar stages through adulthood). In addition to the negative effects of diatom PUAs on copepod fecundity and offspring survival, antiproliferative effects on other invertebrates have also been explored. Larvae of the chordate invertebrate filter-feeder, *Ciona intestinalis*, developed malformed bodies in response to

dissolved PUAs (Lettieri et al., 2015). *Paracentrotus lividus* (sea urchin) embryos in the presence of 2E,4E-decadienal displayed an increased number of abnormalities and deformations as larvae (Romano et al., 2010). Furthermore, 2E,4E-decadienal caused larval development to stop at the gastrula stage and induced considerable numbers of dead or prematurely hatched urchin young.

PUAs can also have toxic effects on vertebrate cells, including cancerous cells (Martinez Andrade et al., 2018). For example, PUAs exhibited anti-proliferative activity and activated programmed cell death (apoptosis) for cancer cells originating from human adenocarcinoma colon and lung cell lines (Sansone et al., 2014). 2E,4E-decadienal also caused the mitochondria of rat liver cells to experience dysfunction and oxidative stress (Sigolo et al., 2008). These observed negative effects of PUAs on vertebrate cells suggest that PUAs may also interact with the developing planktonic offspring of marine vertebrates (Falkowksi, 2002; Nelson et al., 1995).

Marine forage fishes are a critically important vertebrate group in many marine ecosystems. They are indicators of ecosystem health, and every life history stage serves as a resource for higher-order trophic levels (Pikitch et al., 2014). Many species of forage fish spawn in nearshore environments, and their larval stages use the nearshore environment as a nursery ground to feed and develop (Pentilla, 2007). As such, they spend a good portion of their early development sympatric with nearshore diatoms, including both benthic and pelagic forms (Blaxter & Halliday, 1963; Loosanoff, 1938). Forage fishes are planktivorous throughout life, which makes them a vital link in the upward transport of nutrients in marine food chains (Cury et al. 2000, 2003; Freon et al. 2005; Bakun et al. 2010). Forage fishes are a primary food source for many different marine organisms, from sea birds and larger predatory fishes to marine mammals (Butler et al. 2010; Crawford & Dyer 1995; Jahncke et al. 2004; Logan et al. 2011; Magnussen

2011; Pauly et al. 1998; Thompson et al. 1996; Weise & Harvey 2008). Economically, forage fish fisheries generate billions of dollars in revenue and are an important resource for subsistence populations in low-income regions (Palmer et al., 2018; Steiner et al., 2019).

Juvenile stages of forage fishes may be vulnerable to diatom PUAs at several points in their development. During spawning, forage fish females attach their eggs to nearshore substrates that can be heavily populated by diatoms, such as eel grass and macroalgae, moist gravel, and sand (Prazukin et al., 2022, Jewson et al., 2006; Jewson et al., 2006; Smigielski et al., 1984; Quinn et al., 2012). This spawning behavior places embryos in close proximity to benthic diatoms, providing opportunities for interaction between PUAs, developing embryos, and eventually larvae (Figure 1). The possibility for interaction with diatom PUAs is further supported by evidence that benthic diatoms are capable of PUA production at similar concentrations to that of their pelagic counterparts (Pezzolesi et al., 2017). In addition to the potential for early exposure to PUAs via benthic diatoms, another route of exposure may occur post hatch, when larvae can readily ingest pelagic phytoplankton as first-feeding larvae (Lebour, 1921; Blaxter, 1965; Purcell & Grover, 1990; Spittler et al., 1990; Friedenberg et al., 2012).

Determining whether PUAs affect the embryogenesis, development, and survival of early life-history stages of fishes is a logical next step in understanding the role of PUAs in aquatic ecosystems. As a first step toward achieving this objective, in this study I tested for the effects of PUAs on the development of a model vertebrate, the zebrafish, *Danio rerio*. Important advantages of using this model organism are the breadth of information available, their high fecundity, the ease with which they may be bred in the lab, and their rapid development (Urushibata, 2021). Although they are a tropical, freshwater fish, methods for examining zebrafish embryogenesis under toxicity are well-established (Brannen et al., 2010).

Toxicological study of zebrafish provides a straightforward way to estimate how diatom-derived PUAs may affect marine forage fish development.

METHODS

Overview

In order to test for effects of PUAs on a model aquatic vertebrate, zebrafish were exposed to a range of dissolved PUA concentrations and assessed for effects on the biometrics that I describe below. I tested hypotheses based on the following questions.

1. ***Will dissolved PUAs have a negative effect on physiological processes in zebrafish larvae?***

   $\boldsymbol{H_1}$**:** The average heart rate (beats per minute) of zebrafish embryos will be lower in PUA treatments when compared to a control.

   $\boldsymbol{H_0}$**:** The average heart rate (beats per minute) of zebrafish embryos in PUA treatments will not be different when compared to controls.

2. ***Will dissolved PUAs have a negative effect on overall zebrafish standard length, depth, and myotome height at the anus?***

   $\boldsymbol{H_2}$**:** Exposure of embryonic zebrafish to dissolved PUAs will result in reductions in larval length, depth, and myotome height at the anus.

   $\boldsymbol{H_0}$**:** Exposure of embryonic zebrafish to dissolved PUAs will not result in reductions in larval length, depth, and myotome height at the anus.

3. ***Will dissolved PUA exposure cause increased larval zebrafish mortality?***

   $\boldsymbol{H_3}$**:** Exposure of embryonic and larval zebrafish to dissolved PUAs will result in a lower probability of pre-feeding larval survival.

$\boldsymbol{H_0}$**:** Exposure of embryonic and larval zebrafish to dissolved PUAs will not result in a lower probability of survival for pre-feeding larvae.

Animal care

AB wildtype zebrafish were maintained and bred in the lab of Dr. James Cooper at Western Washington University's (WWU) Department of Biology. The animal care protocol for this study was approved by WWU's Institutional Animal Welfare and Use Committee (IACUC), Bellingham, USA, in June of 2021.

PUA treatments

Mixtures of three PUAs, 2E,4E-decadienal ($C_{10}H_{16}O$, Cas No. 25152-84-5), 2E,4E-octadienal ($C_8H_{12}O$, Cas No. 30361-28-5; 5577-44-6), and 2E,4E-heptadienal ($C_7H_{10}O$, Cas No. 4313-03-5) were used to explore the effects of PUAs on zebrafish. The decision to use proportional and increasingly concentrated mixtures of these PUAs, which are heretofore referred to as heptadienal, octadienal, and decadienal, was respectively based on Ruocco et al. (2019). They found that the effects of binary and ternary combinations of these three PUAs on the development of *Paracentrotus lividus* urchin embryos to be synergistic, with negative effects increasing in exposures to combined rather than individual PUAs (Ruocco et al., 2019). This fact, and the knowledge that these three PUAs commonly co-occur *in situ*, led to the decision to use these mixtures rather than individual PUAs (Bartual et al., 2020; Vidoudez et al., 2011).

PUA mixtures were dissolved in methanol, which increases the miscibility of lipid chains (Ribalet et al., 2007; Varella et al., 2014), and homogenized in zebrafish embryo water. While

methanol is a known toxin for vertebrates at various concentrations, it has been found to be less toxic for zebrafish at low concentrations than dimethyl sulfoxide (DMSO), another solvent commonly used in toxicological and developmental work with zebrafish (Christou et al., 2020). Methanol showed no toxicity up to 1% concentration, whereas DMSO showed toxicity at or above 0.3% (Christou et al., 2020). Methanol was therefore favored over DMSO for use in this study.

Dissolved PUA mixture proportions within the range of those shown to be effective at causing developmental difficulties for urchin larvae in Ruocco et al. were used for a preliminary experiment with 24 hours post-fertilization (hpf) zebrafish embryos (decadienal 0.5 μM, heptadienal 1.0 μM, octadienal 1.5 μM; 2019) (Table 1). The proportion of decadienal added to the most concentrated mixture (high) was also within the range of decadienal concentrations shown to compromise the reproduction of the chordate invertebrate *Ciona intestinalis* (0.35, 0.4, 0.45 μg/ml; Lettieri et al., 2015). No change in mortality or morphology of zebrafish larvae was observed at any preliminary concentration after 6-days of exposure. As such, PUA mixture concentrations were increased to levels where these quantifiable effects could be observed (Table 1).

Data collection and statistical analysis

Embryonic heartrate

Embryos were assessed for heartrate changes after exposure to a range of PUA concentrations (H_1) (Table 1). Zebrafish embryos (25) at 24 hpf were placed in plastic petri

dishes and constantly exposed to high, middle, and low concentrations of dissolved PUA mixtures in triplicate for 2 days (Table 1; Figure 2). Water changes were done on both days to account for PUA evaporation and to keep zebrafish waste products and bacterial and fungal levels down. For post-hoc detection of potential methanol effects, a 1% methanol control was added in addition to the embryo water control. Incubation temperatures were set at 28°C, a temperature commonly used for zebrafish rearing (Urushibata et al., 2021). At three days post-fertilization (dpf), ten randomly chosen embryos from each petri dish were video recorded for ten seconds through an Olympus SZ-CTV dissecting microscope mounted with a FLIR Blackfly USB3 Vision camera running the Micro-Manager 2.0.0 program. Heart rate beats per minute (bpm) for each embryo were determined by visually assessing contractions of the heart through the translucent chorion and epidermis. Post-measurement, live embryos were humanely euthanized via a ten-minute submersion in 0°C water before proper disposal.

To account for variance within treatments, a linear mixed model (LMM) was employed and run using R programming version 4.2.2 (lme4 and MuMin packages) to predict heart bpm under PUA treatments. REML was used to make unbiased estimates of variance and covariance parameters and nlminb optimizer to optimize a function with multiple arguments. The model also included variance introduced from petri dish replicates within treatments as a random effect. To assist with model fit, the highly variable dishes were also weighted using the varIdent function, which led to an improvement based on compared Akaike information criterion (AIC) values. For this and following morphometric LMMs, standardized parameters were obtained by fitting the model on a standardized version of the dataset. Confidence Intervals (95%) and p-values were computed using a Wald t-distribution approximation.

Larval morphometrics

To test hypothesis H_2, the same experimental setup was revisited, this time exposing embryos and larvae for 6 days (Table 1; Figure 2). This time frame allowed for 100% hatching. After a 6-day PUA incubation, larvae were humanely euthanized as described above and used in morphometric analyses. For photography, larvae were positioned laterally under a dissecting microscope in a dilute glycerol solution using LAS V4.13 from the Leica Application Suite. ImageJ was used to measure the standard length (the tip of the snout to the end of the spine), and two different dimensions of depth (deepest point of belly and myotome height at anus) of each larval photograph (Figure 3).

For statistical analysis, morphometric responses were first evaluated on an individual basis using the same R packages as for the heart rate experiment before being combined for a multivariate approach. The strength and significance of changes in each distance was determined using similar LMMs appropriate for nested designs. Following that, a principal components analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA) were used to assess differences in approximate larval size between PUA treatments (R, vegan and MVN packages). The PERMANOVA was used due to the data's departure from assumptions of multivariate normality and homogeneity (Anderson, 2017). To visualize how the approximate size of the larvae changed with each PUA increase, a PCA was performed.

Because all morphometric data were collected in one experiment, identical LMMs were used to describe all morphometric measurements (Figure 3). Also, because it had the same design as the heart rate experiment, the same LMM formula addressed the nested design and

variance needs of the data, with models containing petri dishes as a random effect, and weighted dishes leading to improved model fit based on compared AIC values (lme4 and MuMIn packages).

Pre-feeding larval survival

To test H_3, that pre-feeding larval survival will be reduced by PUA exposure, zebrafish embryos were treated with PUAs at 2 hpf. The setup mirrors the previous experiments, with the exceptions that increased numbers of replicates and embryos were included for the sake of statistical power, and total PUA concentrations were decreased for younger embryos (Table 1). Here, four replicate petri dishes were used per treatment with 30 embryos each. Throughout the experiment, larval mortalities were recorded daily until 100% mortality.

Survival probability was assessed using a Kaplan-Meier survival analysis (Jager et al., 2008). The Kaplan-Meier method is a non-parametric survival analysis also called the "time to event" approach. Here it was used to create survival probability curves for zebrafish larvae in PUAs (Di Paolo Ksenia, 2015; Jager et al., 2008). This analysis shows the probability of an event (death) at each time interval (experimental day). The calculations for each survival curve were based on the following equation, which recalibrates the probability of the event of interest at each time interval based on past occurrences (LaMorte, 2016):

$$S_{t+1} = S_t * \left(\frac{N_t + 1 - D_t + 1}{N_t} + 1\right)$$

S_{t+1} = the cumulative probability of surviving beyond time interval *t*,

N_t = the number of subjects at risk at time interval *t*,

D_t = the number of deaths during time interval *t*

The following analyses were carried out using the survminer package in R. First, the Kaplan-Meier equation calculated the survival probability of the zebrafish larvae in each treatment group for each day of the survival experiment, and corresponding survival curves were then generated. A log-rank test for significant differences between curves was implemented, followed by value-adjusted pairwise comparisons to clarify the significant differences between curves.

RESULTS

Heart rate analysis

The intercept for the linear mixed model of heart rate compared to all other treatments, set to the embryo water control treatment, averaged 121.2 bpm. Average larval heart rate decreased significantly by 44 bpm, a 47% reduction, in the high PUA treatment compared to the embryo water control (Table 2). The methanol control and low PUA treatments decreased average larval bpm by 14 and 15 bpm, respectively, but neither difference was found to be statistically different from the control. The explanatory power of the model without random effects, or the proportion of variance explained by the fixed effects relative to the overall variance, was 0.562 (marginal R^2), and was 0.949 when accounting for random effects (conditional R^2) (Figure 4; Table 2). This addition produced a 41% increase in the goodness of fit of the model as indicated by R^2.

Effects of PUAs on larval zebrafish morphometrics

Standard length

The model intercept, or average larval length from the control treatment, was 3.39 mm. The effect of low PUA concentration on larval length was statistically similar to the embryo water control, with an average 0.03 mm decrease in length compared to the embryo water control. This was similar to the 0.03 mm decrease in larval length observed in the methanol only

control (Figure 5). Using this model, the effects of high and moderate PUA treatments were negative and statistically significant. The average standard length of larvae in the high treatment was 2.75 mm, 19% percent smaller than those in the embryo water control ($p = 0.003$). The larvae in the mid PUA treatment were 17% smaller than the control, with an average length of 2.83 mm ($p = 0.006$). When applied to standard length, the total explanatory power of the LMM was 0.88 (conditional R^2), while fixed effects alone, the PUA levels without varying dishes included (marginal R^2) explained 58% of the variation in the data (Table 3). Adding dishes as a random intercept improved the R^2 fit from 0.58 to 0.88.

Deepest vertical distance

The average depths of larvae in the embryo water and methanol control were 0.45 and 0.43 mm, respectively. The effect of the low PUA concentration was non-significant in comparison to the control. The larvae reared in the mid PUA treatment were 23% shallower than control larvae, with an average depth of 0.35 mm (Figure 6). The average depth of larvae in the high PUA treatment was 0.32 mm, 29% percent lower than those in the embryo water control. When applied to deepest vertical distance, the total explanatory power of the LMM was 0.70 (conditional R^2), and the fixed effects alone (marginal R^2) explained 0.48 of the variation in the data (Table 4). Adding dish variation as a random effect improved the R^2 measure of fit by 0.12. Within this model, the effects of high and moderate PUA treatments were negative and statistically significant ($p = 0.001$; $p = 0.006$).

Myotome height at anus

The intercept of the model, set as average myotome height at the anus in the embryo water treatment, was 0.14 mm. The larvae in the high and mid PUA treatments were 0.15 mm on average, 8% percent larger than fish from the embryo water control. The effect of low PUA concentrations was the same as methanol, and non-significant when compared to the control (Table 5). The total explanatory power of the LMM was 0.51 (conditional R^2), and the fixed effects alone (marginal R^2) explained 11% of the variation in the data (Table 5). Adding dishes as a random intercept improved the R^2 fit by 0.40, indicating high variation within treatments. However, unlike the results for length and deepest vertical distance, the effects of high and moderate PUAs on myotome height were not significantly different when compared to the embryo water and methanol control treatments (Figure 7).

Multivariate analysis of larval morphometrics

PUA concentrations caused significant differences in larval sizes and were responsible for 65% of the overall size variation between treatment concentrations (PERMANOVA, $F_{4,322}$, p = < 0.001, $R^2 = 0.65$). Pairwise comparisons showed seven significantly different groups. The difference in larval sizes between the embryo water control and the methanol treated larvae was significant, indicating that 1% methanol may have a small yet statistically significant negative effect on larval zebrafish growth (Table 6). The effect of low PUAs on size was not significantly different to the effect of methanol alone. The larvae from the high and mid PUA concentrations

were significantly different in size compared to both the low PUA and methanol treatments ($p = 0.01$), but not significantly different from each other. Therefore, although methanol had a small effect on size ($p = 0.01$), the effects must be partially due to PUAs because larvae in the mid and high PUA treatments were significantly different in size to those in the methanol treatment.

A PCA of the multivariate dataset calculated 1st and 2nd principal components that accounted for 93% of the total variation in the data (~59% and ~34%, respectively) (Table 7). Ordination of PCs 1 and 2 shows the size differences between treatment groups of larvae based on the three morphological measurements used in the analysis (Figure 8). Larvae from the control, methanol, and low PUA treatments grouped closely on PC1, indicating similarity in overall size (Figure 8). Larvae from the mid and high PUA treatments were also closely associated, but spaced farther apart in length and depth, showing that not all larvae in these higher concentrations were uniformly affected. Additionally, a non-metric dimensional scaling analysis confirmed the strength of the group patterns and their positioning by converging on a similar solution after 20 iterations, with an acceptable stress value of 0.05.

Pre-feeding survival

Some larvae in the embryo water and methanol treatment had survivors until day 15, while larvae in low and mid PUA treatments did not live beyond day 12. Over 75% of larvae in the high PUA treatment died after 1 day of exposure, and nearly 100% had expired by day 5 (Figure 9). None of the larvae in high PUAs survived to the transition stage where yolk absorption changes to exogenous feeding (~8 dpf). A log-rank test of the survival curves indicated that significant differences in survival probabilities between the PUA treatments ($p = < 0.001$). Bonferroni-adjusted pairwise comparisons showed that the two control groups survived

at similar rates (Figure 9; Table 8). The survival probability for fish in low PUAs was significantly different when compared to either control, while the difference between fish in the low and mid PUA concentrations were statistically non-significant. In the medium treatment, the survival probability was significantly different compared to both controls and to the high treatment. The highest PUA treatment had a survival probability that was significantly different from all other treatments.

DISCUSSION

This study is the first to show that diatom specific PUAs can negatively affect the embryonic and larval development of a fish species. I found that PUAs reduced the heart rate, overall size, and pre-feeding survival of embryonic zebrafish. Findings presented here suggest that diatom derived PUAs are likely to impair the development of marine forage fishes. In the following sections I will discuss the implications of these findings on zebrafish, and assuming that observations are transferable to forage fishes, I will discuss the implications for the fitness of marine fishes exposed to PUAs during development.

Heart Rate

Zebrafish embryos exposed to higher PUA concentrations had lower heart rates than those in control treatments (Figure 4; Table 2). Embryonic fish hearts are particularly vulnerable to environmental toxins, such as PUAs, due to their highly permeable epithelia and the absence of organs responsible for blood filtration (e.g., liver and kidneys) that have yet to develop (Incardona & Scholz, 2017). Vertebrates exposed to oxylipins, such as PUAs, are known to develop atherosclerosis, experience problems with platelet aggregation and vascular constriction, and to be prone to cardiac injury (Caligiuri et al., 2017; Nayeem, 2018). Embryonic zebrafish hearts should be especially susceptible to the effects of PUA toxicity due to the ease with which these toxins can reach heart tissues and the lack of compensatory mechanisms that will arise in later

development. Therefore, it is reasonable to assume that PUAs will have similar effects on embryonic fishes and their cardiac health when they are exposed to PUAs in nature.

The consequences of early cardiac injury in fishes can be catastrophic. For example, the lake trout, *Salvelinus namaycush*, (Artedi et al., 1792) population of the Great Lakes collapsed because of heart-related issues caused by the exposure of trout embryos to the industrial pollutant dioxin (Cook et al., 2003). Chemical exposure to dioxin in lake trout caused symptoms similar to those that were seen in zebrafish exposed to PUAs. For lake trout, stress from dioxin presents first as pre-hatch heart abnormalities before progressing into problems with circulatory dysregulation, anemia, and hypoxia, eventually resulting in premature death from attendant lesions in the brain, retina, liver, and other organs (Spitsbergen et al., 1991). This circulatory dysregulation was also shown to cause yolk sac edema and impair swim bladder development (Guiney et al., 1997; Lanhametal et al., 2014; Spitsbergen et al., 1991). I observed anecdotally that zebrafish treated with PUAs frequently exhibited cardiac edema and underdeveloped swim bladders, which suggests that these fish may have experienced circulatory dysregulation symptoms beyond decreased heart rate. If the embryonic and larval stages of forage fishes are exposed to the PUA concentrations used in this study, then it is plausible that they would experience similar, life-threatening disruptions to circulatory development.

Morphometrics

PUA exposure affected the morphological development of zebrafish by reducing body length, body depth, and overall size (Figure 5-8; Table 3-6). If the mechanisms which retard zebrafish growth after PUA exposure are similar in forage fishes, then PUA exposure may affect

forage fish fitness through multiple pathways. For example, larval fish survival is coupled with larval body size, with smaller larvae experiencing higher rates of predation (Cowan et al., 1996; Paradis, 1996). For any planktonic organism the risk of predation is significant, and this is also true for forage fishes, where predation is one of the most important causes of mortality (Miller et al., 1988; Bailey & Houde, 1989; Paradis, 1996; Peterson & Wroblewski, 1984). As such, high selective pressure exists on planktonic organisms, including forage fishes, to grow rapidly. If through PUA exposure causes forage fishes to grow at a slower rate, then this increases their exposure to predation which can eventually affect recruitment success (Houde, 2008).

Smaller fishes also tend to have slower swimming speeds and lower feeding efficiencies in comparison to larger conspecifics (Houde, 2008; Gleason & Bangston., 1996; Houde & Schekter, 1980; Hare & Cowen, 1997). In bay anchovies (*Anchoa mitchilli*; Valenciennes, 1848) feeding efficiency has been shown to increase with size (Gleason & Bangston., 1996; Houde & Schekter, 1980). In bluefish (*Pomatomus saltatrix*; Linnaeus, 1766) larger size was correlated with higher survival rates from yolk-sac-stage embryos to first-feeding larvae (Hare & Cowen, 1997). Raventos and MacPherson (2005) also found that larval size was an accurate predictor of post-settlement survival for two species of reef fishes, the five-spotted wrasse (*Symphodus roissali;* Risso, 1810*)* and the ocellated wrasse *(Symphodus ocellatus; Linnaeus, 1758).*

The observation that fish larvae experience slower swimming speeds and reduced feeding efficiencies relative to later life stages is likely a result of the relationship between larval size and fluid dynamics (Van Leeuwen et al., 2015; Voesenek et al., 2018). After hatching most larval fishes continue to develop by way of yolk and lipid reserves provided by their mothers (Kamler, 2008). They quickly develop functional mouth parts, allowing them to feed and acquire energy exogenously (Yúfera & Darias, 2007).

Larval stages of fishes, including forage fishes, encompass a size range that places them in an intermediate hydrodynamic regime between laminar (viscous) and turbulent (inertial) flow (Voesenek et al., 2018). The relative dominance of viscous and inertial forces is estimated by calculating the Reynolds number (Van Leeuwen et al., 2015). Larval sizes < 5mm, which encompasses the sizes of zebrafish measured here, and those of most larval forage fishes, places them in a low Reynolds number environment in which viscous forces dominate (Van Leeuwen et al., 2015). Because movement through more viscous fluids requires a higher cost of transport (Schmidt-Nielson, 1972), and because higher viscosities lower maximum swimming speeds and reduce a larvae's ability to avoid predators, prolonging the time that young fishes spend in a low Reynolds number environment can greatly reduce survival (Voesenek et al., 2018). Further, it is a taxation on endogenous energy reserves. If these reserves are exhausted before transition to exogenous feeding, starvation is likely to result (Yin & Blaxter, 1987).

Existing in a high viscosity environment also requires small larval fishes to employ higher forces (relative to body size) when suction feeding, which is usually the only feeding strategy available for them (China & Holzman, 2014; Cooper et al., 2020). The ratio of energy expended to energy acquired during feeding will therefore be higher for smaller fishes. Additionally, the movement required by small fish larvae in viscous environments requires the beating of their tails at a high frequency, which in turn is dependent on the use of fast-twitch muscles. These muscles are fueled by mitochondria-dense fibers with a high ATP demand and energy reserves (Huriaux et al., 1996; Van Leeuwen et al., 2016). Prolonged residence in a high-viscosity environment increases the likelihood of starvation for larval fishes, which is already the largest source of mortality for most larval fishes (Hjort, 1914; Houde, 2002).

Pre-feeding survival

In experiments where pre-feeding larvae were exposed to PUAs and reared until starvation, PUA exposure accelerated rates of larval mortality in comparison to control treatment larvae (Figure 9; Table 8). Most specimens exposed to high PUA concentrations did not survive until they would otherwise have begun to feed exogenously, and mortality rates were higher for fish from the low and medium PUA treatments in comparison to the controls (Figure 9; Table 8). The inference that PUAs impose general stress to developing fishes resulting in reductions in survival is supported by my finding that PUAs affected zebrafish embryonic heart rates, and by extension circulatory dysregulation and the associated damaging effects (Caligiuri et al., 2017; Nayeem, 2018). The implications of this finding are significant for the ecology of forage fish larvae. The added physiological stress imposed by PUA exposure may exacerbate starvation stress and reduce survival in larval forage fishes.

Conclusions and future directions

This study provides the first evidence that diatom derived PUAs can negatively affect the development of fishes. This is also the first study to explore the effects of PUAs on a vertebrate. Given the sympatry of diatoms with embryonic and larval stages of forage fishes in most temperate marine ecosystems, and the clear effects of PUAs on a larval fish, the likelihood that PUAs will negatively affect the fitness of forages fishes seems high. If true, then this pathway represents an unexplored mechanism that might explain some of the variation in interannual variability in forage fish population dynamics. My findings also suggest that there are pathways

for diatom-derived PUAs to affect marine food webs and marine secondary production beyond the diatom-copepod link.

While not quantified in this study, zebrafish embryos in the size and mortality experiments tended to hatch later if they had been exposed to high PUA concentrations, and many needed assistance (manually rupturing the chorion) in order to be released from their eggs. These observations suggest that the hatching success of embryos born in areas of high diatom density might be compromised. As such, hatching success might be a useful metric for quantifying the effects of PUAs on the development of fish species. My findings also suggest that future experiments could include other meaningful markers of PUA stress on fitness like the number of edematous larvae, swim bladder development, and later life history stages. Given the clear potential presented here for negative interactions between PUAs and developing fish, especially forage fishes, further exploration of the effects caused by PUA exposure will likely add to our current understanding of the nearshore environmental ecology of forage fishes and diatom PUAs and its implications.

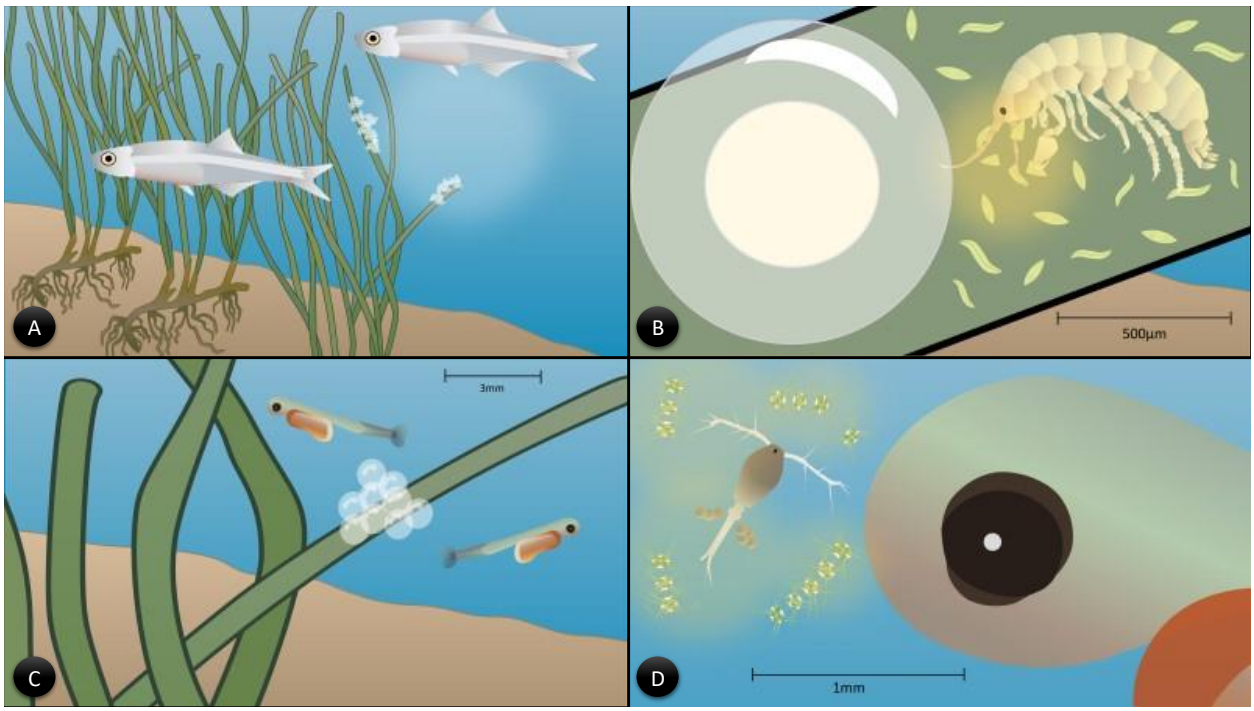

Figure 1. Pathways for embryonic and larval forage fish interactions with PUAs. A) A forage fish nearshore spawning event where eggs are deposited on substrate harboring benthic diatoms. B) Embryos experiencing PUA exposure from benthic diatoms releasing PUAs upon grazing-induced damage to diatom cell walls. C) Early-stage larval forage fish maturing near hatch location with continued exposure to dissolved PUAs. D) First-feeding larval forage fish experiencing PUA exposure indirectly through pelagic zooplankton grazing and directly via ingestion of diatoms and zooplankton.

Table 1. Total PUA mixture concentrations (µg/ml) and individual PUA proportions (amount added) for all experiments (Total = total PUAs in µg/ml).

PUA Mixture Concentrations and Composition (µg/ml)				
Preliminary Experiment	**PUA**	**Low**	**Mid**	**High**
Amount added:	Octadienal	0.12	0.56	1.12
	Heptadienal	0.06	0.33	0.66
	Decadienal	0.04	0.19	0.38
	Total	**0.22**	**1.08**	**2.16**
Morphometrics and Heart Rate Experiments	**PUA**	**Low**	**Mid**	**High**
	Octadienal	1.12	5.59	11.18
	Heptadienal	0.66	3.30	6.61
	Decadienal	0.38	1.90	3.81
	Total	**2.16**	**10.80**	**21.59**
Survival Experiment	Octadienal	1.12	2.24	3.36
	Heptadienal	0.66	1.32	1.98
	Decadienal	0.38	0.76	1.14
	Total	**2.16**	**3.24**	**6.48**

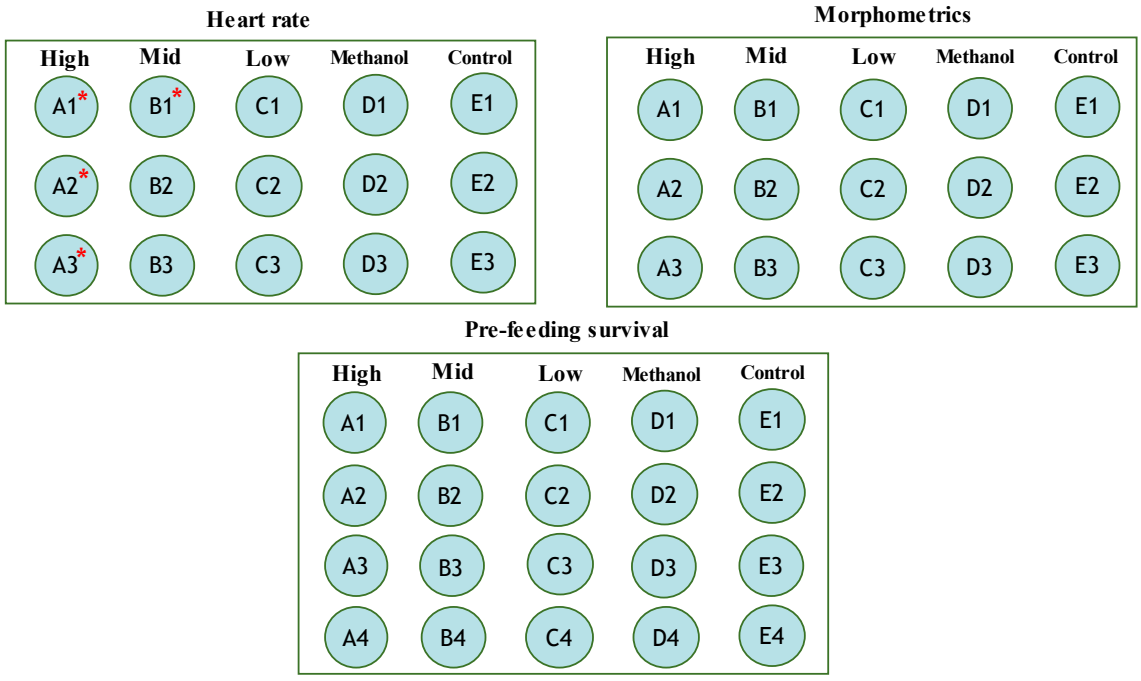

Figure 2. Design for 3 nested developmental experiments to test for PUA effects on zebrafish larvae. Blue circles represent petri dishes housed in an environmental incubator. Control = embryo water control; Methanol = embryo water plus methanol solvent. Larval morphometrics, 25 embryos per plate, top right; Heart rate, 25 embryos per plate, top left (red * = dish expired before recording); Survival, 30 embryos per plate, bottom.

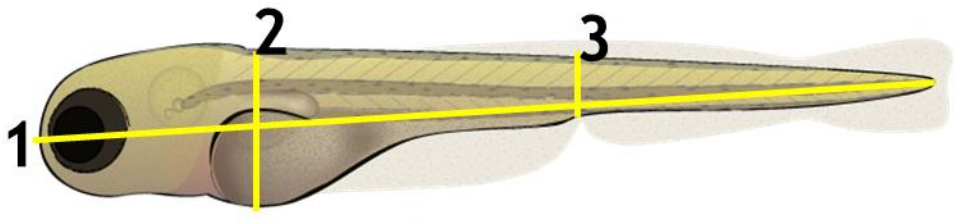

Figure 3. The three dimensions measured for larval fish morphometrics: Line 1 = standard length; Line 2 = deepest vertical point; Line 3 = myotome height at anus (Image Credit: Lizzy Griffiths)

Table 2. Linear mixed model results for heart rate in beats per minute (Bpm) of 3 dpf zebrafish larvae after constant PUA exposure at 2 different concentrations (Table 1). (Intercept) = Control treatment (R, lme4, sjPlot and MuMIn packages). Bolded values indicate statistically significant p-values.

	Bpm		
Predictors	*Estimates*	*CI*	*p*
[Control] (Intercept)	121.20	106.83 – 135.57	**<0.001**
Treatment [Methanol]	-15.00	-39.22 – 9.22	0.187
Treatment [Low]	-14.20	-38.42 – 10.02	0.208
Treatment [High]	-44.10	-71.18 – -17.02	**0.006**
Random Effects			
σ^2	141.71		
$\tau_{00\ DishNumber}$	143.26		
$N_{\ DishNumber}$	11		
Observations	110		
Marginal R^2 / Conditional R^2	0.562 / 0.949		

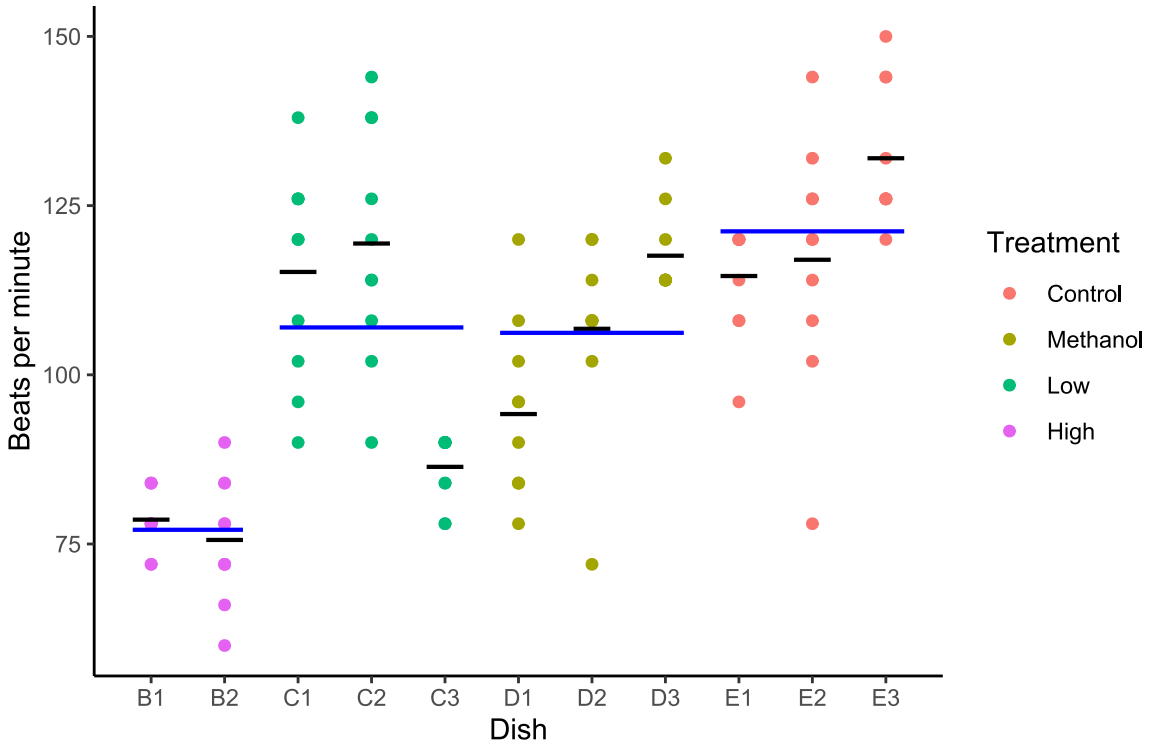

Figure 4. Larval heart beats per minute under PUA exposure. PUA treatments are differentiated by color. Petri dishes are labeled 1-3 on the x-axis and have letters and numbers representing their treatments and replicates (Figure 2). Blue bars indicate treatment averages and black bars represent dish averages. Dishes A1-3 and plate B3 expired on day 1 and data are not available.

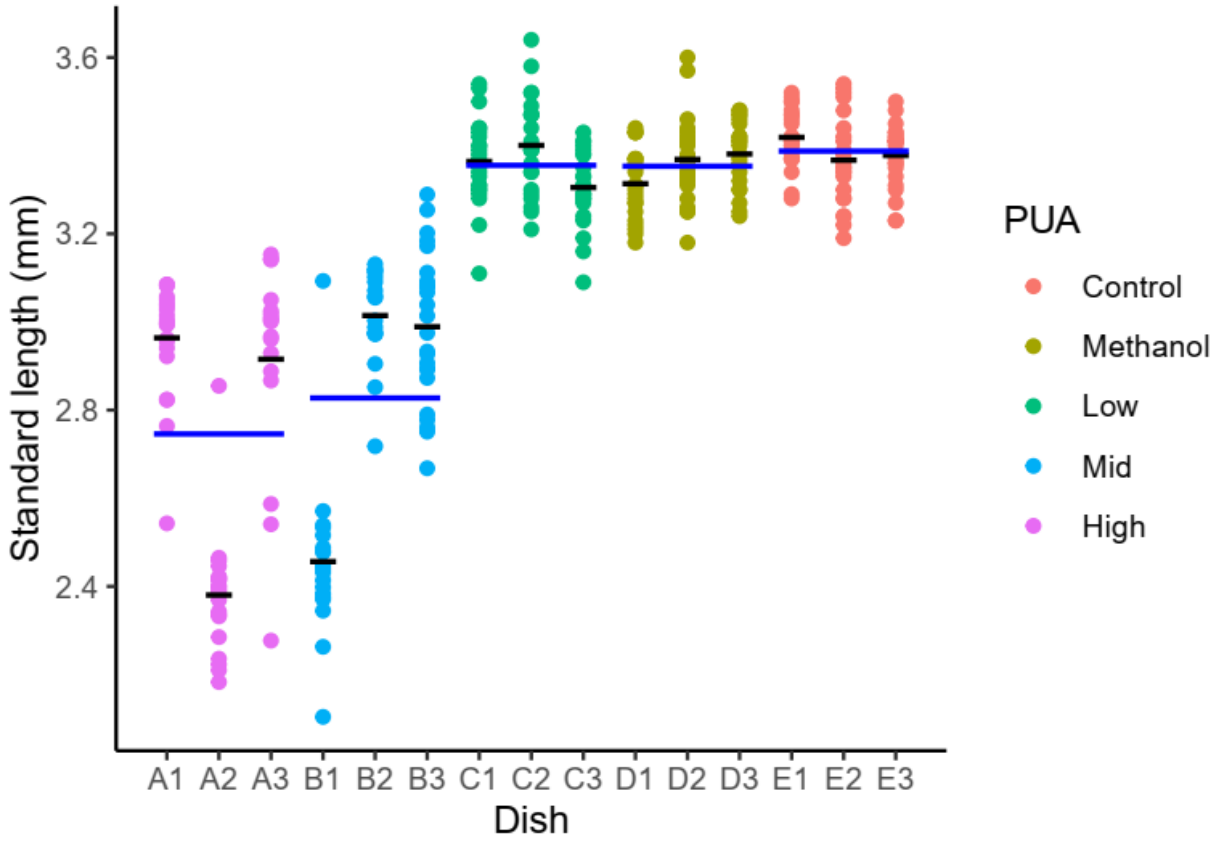

Figure 5. Larval standard length under PUA exposure. PUA concentrations are differentiated by color. Length is in mm on the y-axis. Individual replicate petri dishes for each treatment are labeled A1-E3 on the x-axis with letters representing concentration (A = High, B = Mid, C = Low, D = Methanol, E = Control). Blue bars indicate treatment averages and black bars within dish averages.

Table 3. Linear mixed model results for standard length (Length) of zebrafish 7 dpf larvae after 6 days of constant PUA exposure at 3 different concentrations (Table 1). All groups were compared to the intercept, which in this model is the average from the control treatment. Bolded values indicate statistically significant p-values (R, lme4, sjPlot and MuMIn packages).

	Length		
Predictors	*Estimates*	*CI*	*P*
[Control] (Intercept)	3.39	3.16 – 3.62	**<0.001**
Treatment [Methanol]	-0.03	-0.40 – 0.33	0.841
Treatment [Low]	-0.03	-0.40 – 0.33	0.851
Treatment [Mid]	-0.57	-0.93 – -0.20	**0.006**
Treatment [High]	-0.64	-1.00 – -0.27	**0.003**
Random Effects			
σ^2	0.02		
$\tau_{00\ DishNumber}$	0.04		
ICC	0.71		
$N_{\ DishNumber}$	15		
Observations	326		
Marginal R^2 / Conditional R^2	0.585 / 0.879		

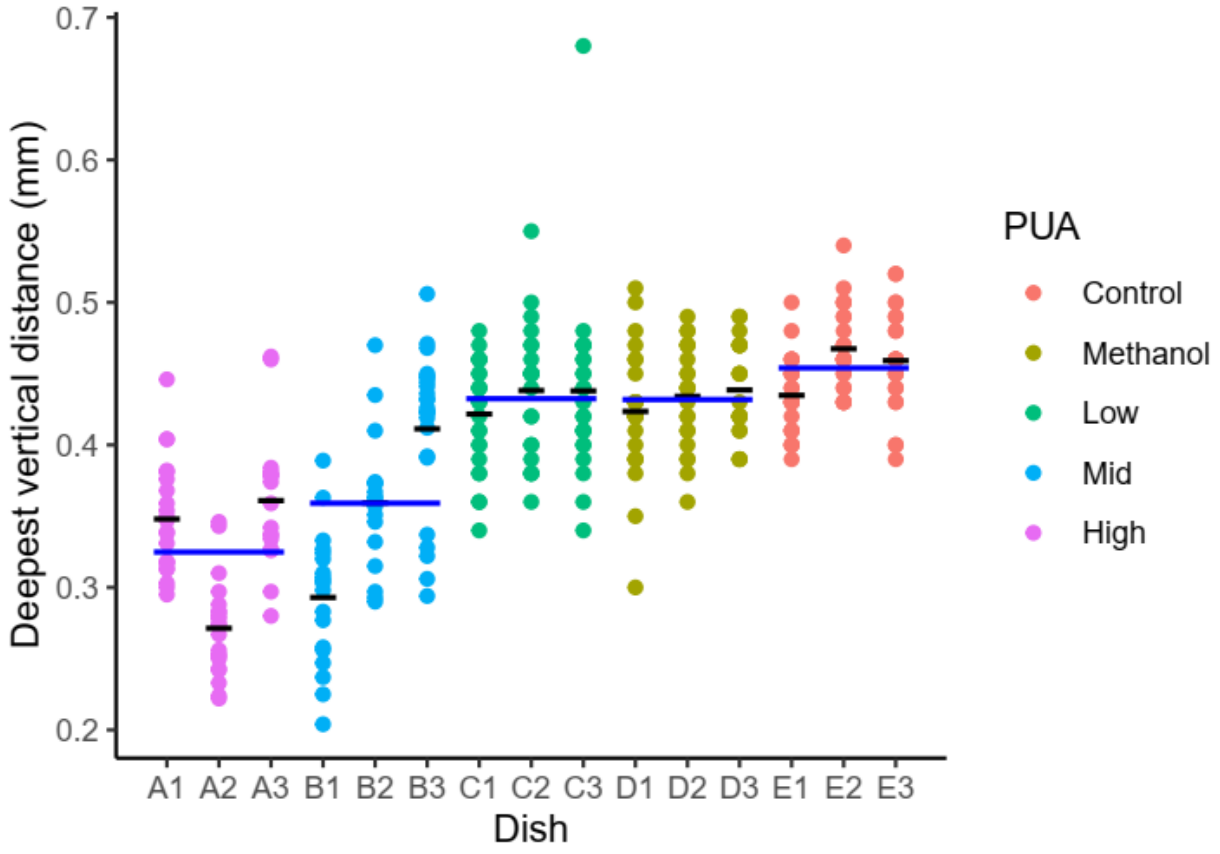

Figure 6. Deepest vertical point (Figure 3; Depth 2) for zebrafish larvae exposed to PUAs. PUA concentrations are differentiated by color. Depth is in mm on the y-axis. Petri dishes are labeled A1-E3 on the x-axis and have letters and numbers representing their concentration and replicates (A = High, B = Mid, C = Low, D = Methanol, E = Control). Blue bars indicate group averages and black bars within dish averages.

Table 4. Linear mixed model results for deepest vertical distance (Depth 2) of 7 dpf zebrafish larvae after constant PUA exposure at 3 different levels (Table 1). All groups were compared to the intercept, which in this model the control treatment (R, lme4, sjPlot and MuMIn packages).

	Depth 2		
Predictors	*Estimates*	*CI*	*P*
[Control] (Intercept)	0.45	0.41 – 0.49	**<0.001**
Treatment [Methanol]	-0.02	-0.08 – 0.04	0.463
Treatment [Low]	-0.02	-0.08 – 0.04	0.471
Treatment [Mid]	-0.10	-0.16 – -0.04	**0.006**
Treatment [High]	-0.13	-0.19 – -0.06	**0.001**
Random Effects			
σ^2	0.00		
$\tau_{00\ DishNumber}$	0.00		
ICC	0.43		
$N_{\ DishNumber}$	15		
Observations	326		
Marginal R^2 / Conditional R^2	0.477 / 0.701		

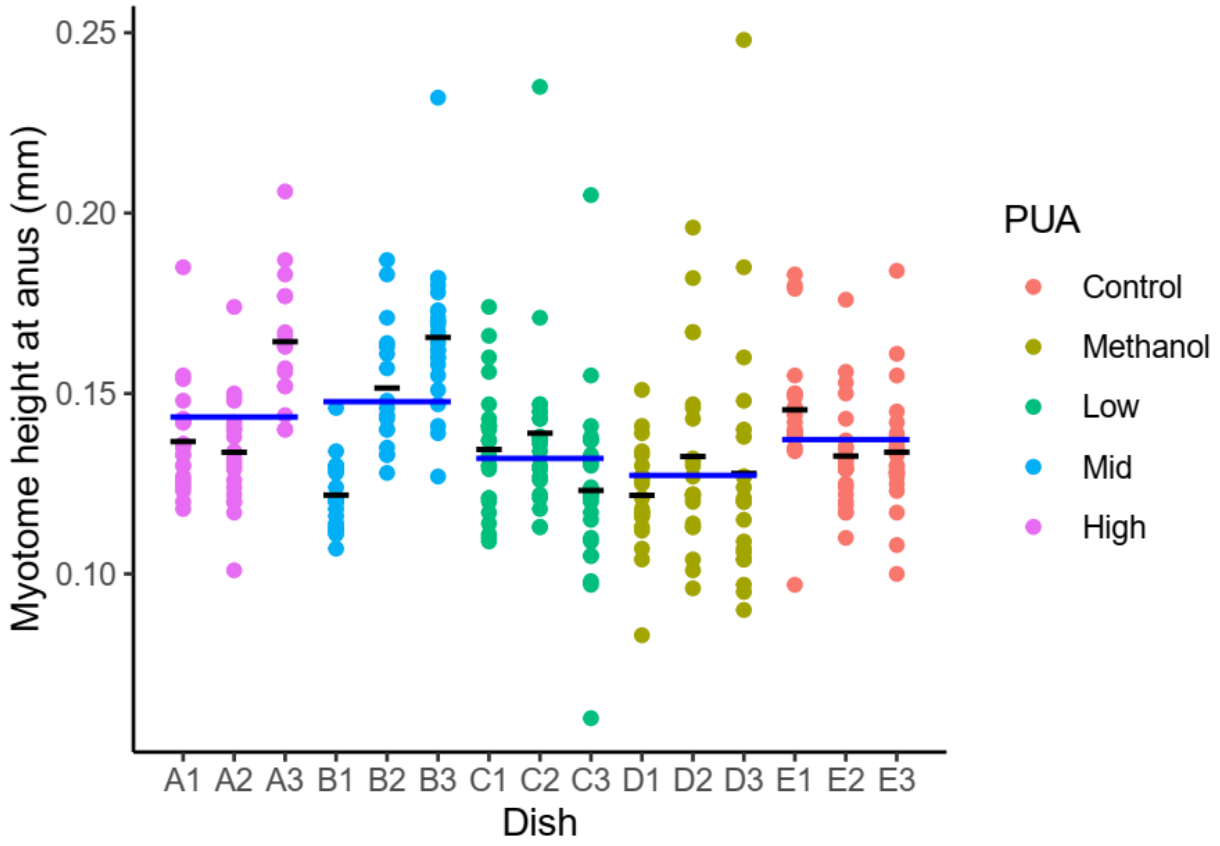

Figure 7. Myotome height at the anus (Depth 3). PUA levels are differentiated by color. Measurements are in mm on the y-axis. Petri dishes are labeled A1-E3 on the x-axis. Letters and numbers represent concentration and replicate (A = High, B = Mid, C = Low, D = Methanol, E = Control). Blue bars indicate group average and black bars within dish average.

Table 5. Linear mixed model results for myotome height at the anus (Depth 3) of 7 dpf zebrafish larvae after constant PUA exposure at 3 different concentration levels (Table 1). All groups were compared to the intercept, which in this model is the control treatment. Bolded values indicate statistically significant p-values (R, lme4, sjPlot and MuMIn packages).

	Depth 3		
Predictors	*Estimates*	*CI*	*p*
Control (Intercept)	0.14	0.12 – 0.15	**<0.001**
Treatment [Methanol]	-0.01	-0.04 – 0.02	0.402
Treatment [Low]	-0.01	-0.03 – 0.02	0.671
Treatment [Mid]	0.01	-0.02 – 0.03	0.466
Treatment [High]	0.01	-0.02 – 0.03	0.526
Random Effects			
σ^2	0.00		
$\tau_{00\ DishNumber}$	0.00		
ICC	0.45		
$N_{\ DishNumber}$	15		
Observations	326		
Marginal R^2 / Conditional R^2	0.110 / 0.506		

Table 6. PERMANOVA pairwise comparisons with non-adjusted and Bon Ferroni adjusted p-values. Sig = $p < 0.05$ (R, pairwiseAdonis and vegan packages).

Pair	Df	SS	F Model	R2	p-value	p-adjusted	Sig
High vs Mid	1.000	0.009	2.674	0.022	0.083	0.830	
High vs Low	1.000	0.349	186.363	0.593	0.001	0.010	*
High vs Methanol	1.000	0.343	184.448	0.596	0.001	0.010	*
High vs Control	1.000	0.393	221.308	0.634	0.001	0.010	*
Mid vs Low	1.000	0.243	134.487	0.512	0.001	0.010	*
Mid vs Methanol	1.000	0.237	132.489	0.515	0.001	0.010	*
Mid vs Control	1.000	0.279	163.046	0.560	0.001	0.010	*
Low vs Methanol	1.000	0.000	0.288	0.002	0.755	1.000	
Low vs Control	1.000	0.002	5.014	0.035	0.011	0.110	
Methanol vs Control	1.000	0.003	9.461	0.065	0.001	0.010	*

Table 7. Standard deviation, proportion of variance, and cumulative proportion explained by principal components 1 (PC1), 2 (PC2) and 3 (PC3).

	PC1	PC2	PC3
Standard deviation	1.3302	1.0062	0.467
Proportion of Variance	0.5898	0.3375	0.0727
Cumulative Proportion	0.5898	0.9273	1.0000

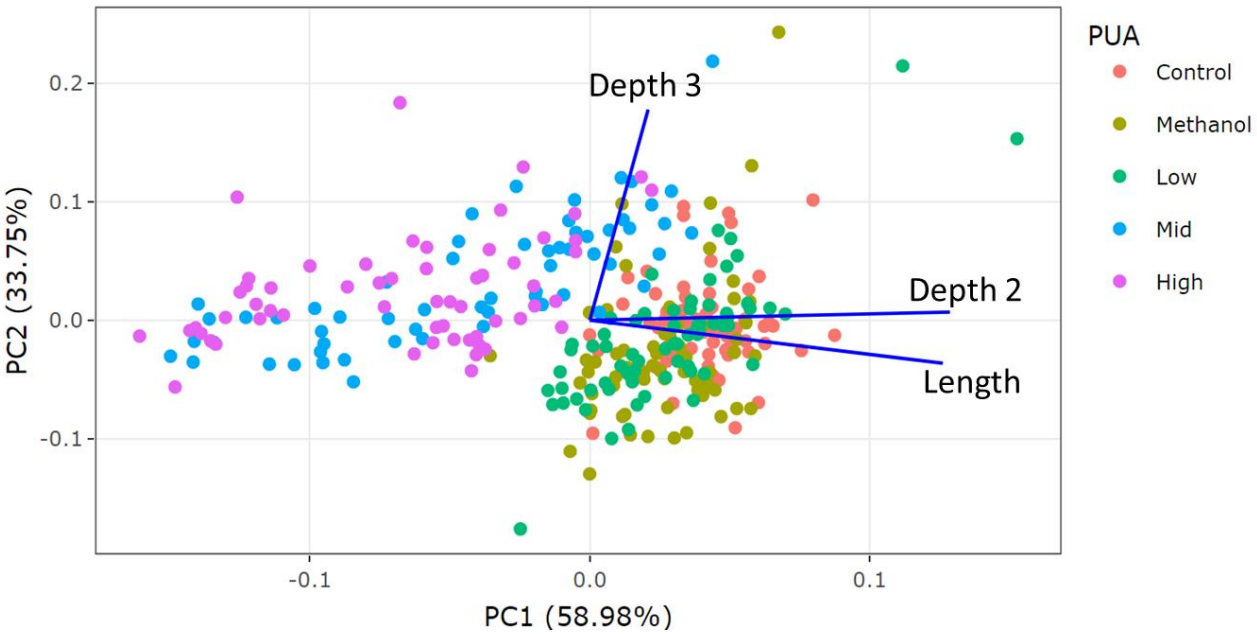

Figure 8. PCA of the variation in size of fish larvae based on PC1 and PC2. Vectors in blue show the influence of the variable on the position of larvae. PC1 increases with Length (standard length) and Depth 2 (deepest point) vectors. Depth 3 (myotome height at anus) increased on PC2. Larger larvae are located toward the top and right and smaller to the bottom left (R, plotly package).

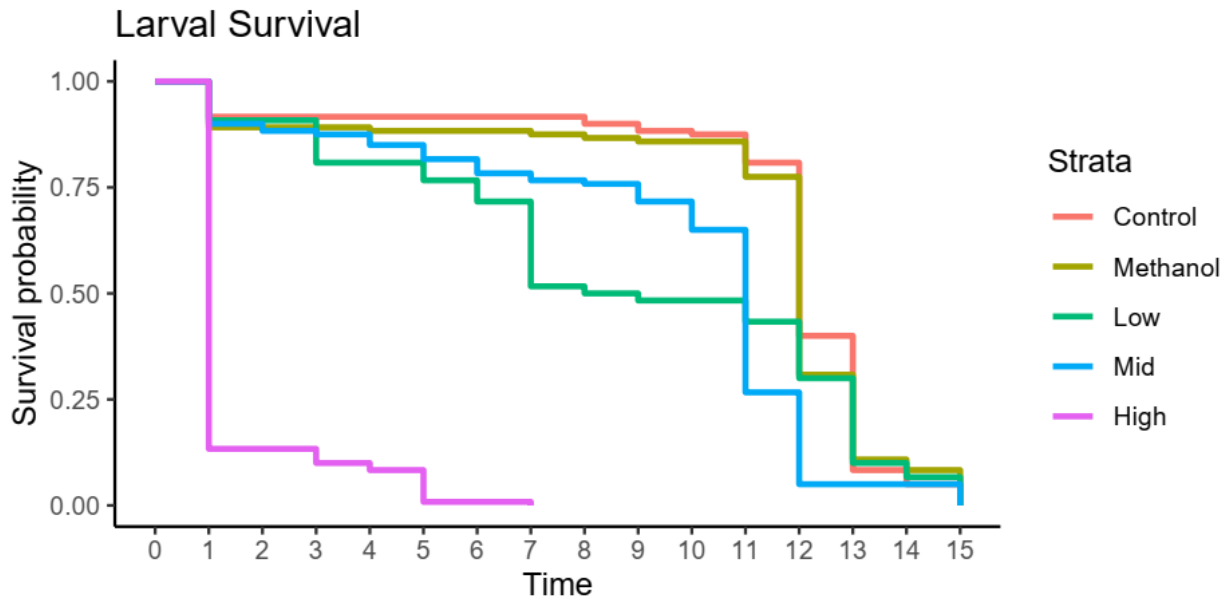

Figure 9. Kaplan-Meier survival probability curves. The y-axis shows the probability of survival as a ratio from 0-1. Three increasing concentrations of PUAs are shown by color, with 2 controls (Control = untreated embryo water; Methanol = 1% methanol solvent).

Table 8. P-values from Bonferroni adjusted pairwise comparisons of 5 different treatment group survival curves in the Kaplan-Meier analysis.

PUA	Low	Mid	High	Control
Mid	0.1742	-	-	-
High	<0.0001	<0.0001	-	-
Control	0.0028	<0.0001	<0.0001	-
Methanol	0.0076	<0.0001	<0.0001	0.5976

Works Cited

Adolph, S., Poulet, S. A., & Pohnert, G. (2003). Synthesis and biological activity of α,β,γ,δ-unsaturated aldehydes from diatoms. *Tetrahedron, 59*(17), 3003–3008. https://doi.org/10.1016/S0040-4020(03)00382-X

Anderson, M. J. (2017). Permutational Multivariate Analysis of Variance (PERMANOVA). In *Wiley StatsRef: Statistics Reference Online* (pp. 1–15). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118445112.stat07841

Artedi, P., Walbaum, J. J., Linné, C. von, & Artedi, P. (1792). *Petri Artedi Sueci Genera piscium: In quibus systema totum ichthyologiae proponitur cum classibus, ordinibus, generum characteribus, specierum differentiis, observationibus plurimis : redactis speciebus 242 ad genera 52 : Ichthyologiae pars III.* Grypeswaldiae : Impensis Ant. Ferdin. Röse. http://archive.org/details/petriartedisueci03arte

Bailey, K. M., & Houde, E. D. (1989). Predation on eggs and larvae of marine fishes and the recruitment problem. In J. H. S. Blaxter & A. J. Southward (Eds.), *Advances in Marine Biology* (Vol. 25, pp. 1–83). Academic Press. https://doi.org/10.1016/S0065-2881(08)60187-X

Bakun, A., Babcock, E. A., Lluch-Cota, S. E., Santora, C., & Salvadeo, C. J. (2010). Issues of ecosystem-based management of forage fisheries in "open" non-stationary ecosystems: The example of the sardine fishery in the Gulf of California. *Reviews in Fish Biology and Fisheries, 20*(1), 9–29. https://doi.org/10.1007/s11160-009-9118-1

Barbosa, M., Valentão, P., & Andrade, P. B. (2016). Biologically active oxylipins from enzymatic and nonenzymatic routes in macroalgae. *Marine Drugs*, *14*(1), Article 1. https://doi.org/10.3390/md14010023

Bartual, A., Hernanz-Torrijos, M., Sala, I., Ortega, M. J., González-García, C., Bolado-Penagos, M., López-Urrutia, A., Romero-Martínez, L., Lubián, L. M., Bruno, M., Echevarría, F., & García, C. M. (2020). Types and distribution of bioactive polyunsaturated aldehydes in a gradient from mesotrophic to oligotrophic waters in the Alborán Sea (Western Mediterranean). *Marine Drugs*, *18*(3), 159. https://doi.org/10.3390/md18030159

Blaxter, J. H. S., & Holliday, F. G. T. (1963). The behaviour and physiology of herring and other Clupeids. In F. S. Russell (Ed.), *Advances in Marine Biology* (Vol. 1, pp. 261–394). Academic Press. https://doi.org/10.1016/S0065-2881(08)60261-8

Bourrachot, S., Simon, O., & Gilbin, R. (2008). The effects of waterborne uranium on the hatching success, development, and survival of early life stages of zebrafish (*Danio rerio*). *Aquatic Toxicology*, *90*(1), 29–36. https://doi.org/10.1016/j.aquatox.2008.07.013

Brannen, K. C., Panzica-Kelly, J. M., Danberry, T. L., & Augustine-Rauch, K. A. (2010). Development of a zebrafish embryo teratogenicity assay and quantitative prediction model. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, *89*(1), 66–77. https://doi.org/10.1002/bdrb.20223

Caldwell, G. S. (2009). The influence of bioactive oxylipins from marine diatoms on invertebrate reproduction and development. *Marine Drugs*, *7*(3), 367–400. https://doi.org/10.3390/md7030367

Caligiuri, S. P. B., Parikh, M., Stamenkovic, A., Pierce, G. N., & Aukema, H. M. (2017). Dietary modulation of oxylipins in cardiovascular disease and aging. *American Journal of Physiology-Heart and Circulatory Physiology*, *313*(5), H903–H918. https://doi.org/10.1152/ajpheart.00201.2017

Ceballos, S., & Ianora, A. (2003). Different diatoms induce contrasting effects on the reproductive success of the copepod *Temora stylifera*. *Journal of Experimental Marine Biology and Ecology*, *294*(2), 189–202.

China, V., & Holzman, R. (2014). Hydrodynamic starvation in first-feeding larval fishes. *Proceedings of the National Academy of Sciences*, *111*(22), 8083–8088. https://doi.org/10.1073/pnas.1323205111

Christou, M., Kavaliauskis, A., Ropstad, E., & Fraser, T. W. K. (2020). DMSO effects larval zebrafish (*Danio rerio*) behavior, with additive and interaction effects when combined with positive controls. *Science of The Total Environment*, *709*, 134490. https://doi.org/10.1016/j.scitotenv.2019.134490

Cowan, J. H., Jr, Houde, E. D., & Rose, K. A. (1996). Size-dependent vulnerability of marine fish larvae to predation: An individual-based numerical experiment. *ICES Journal of Marine Science*, *53*(1), 23–37. https://doi.org/10.1006/jmsc.1996.0003

Crawford, R., & DYER, B. (2008). Responses by four seabird species to a fluctuating availability of Cape Anchovy *Engraulis capensis* off South Africa. *Ibis*, *137*, 329–339. https://doi.org/10.1111/j.1474-919X.1995.tb08029.x

Cury, P., Bakun, A., Crawford, R., Jarre, A., Quiñ, R., Shannon, L., Verheye, H., & Quinones, R. (2000). Small pelagics in upwelling systems: Patterns of interaction and structural changes in "wasp-waist" ecosystems. *ICES Journal of Marine Science*, *57*, 603–618. https://doi.org/10.1006/jmsc.2000.0712

Cury, P., Shannon, L., & Shin, Y.-J. (2003). The functioning of marine ecosystems: A fisheries perspective. *Responsible Fisheries in the Marine Ecosystem*, 103–123. https://doi.org/10.1079/9780851996332.0103

Cuvier, G. (1769-1832) A. du texte, & Valenciennes, A. (1794-1865) A. du texte. (1828). *Histoire naturelle des poissons. Tome 20 / par le Bon Cuvier, Et par M. Valenciennes.* https://gallica.bnf.fr/ark:/12148/bpt6k6365342h

Di Paolo, C., Groh, K. J., Zennegg, M., Vermeirssen, E. L. M., Murk, A. J., Eggen, R. I. L., Hollert, H., Werner, I., & Schirmer, K. (2015). Early life exposure to PCB126 results in delayed mortality and growth impairment in the zebrafish larvae. *Aquatic Toxicology*, *169*, 168–178. https://doi.org/10.1016/j.aquatox.2015.10.014

Falkowski, P., Barber, R., & Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science (New York, N.Y.)*, *281*, 200–207.

Falkowski, P. (2002). The Ocean's Invisible Forest. *Scientific American*, *287*(2), 54–61.

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, *281*(5374), 237–240. https://doi.org/10.1126/science.281.5374.237

Fontana, A., d'Ippolito, G., Cutignano, A., Miralto, A., Ianora, A., Romano, G., & Cimino, G. (2007). Chemistry of oxylipin pathways in marine diatoms. *Pure and Applied Chemistry*, *79*(4), 481–490. https://doi.org/10.1351/pac200779040481

Franzè, G., Pierson, J. J., Stoecker, D. K., & Lavrentyev, P. J. (2018). Diatom-produced allelochemicals trigger trophic cascades in the planktonic food web. *Limnology and Oceanography*, *63*(3), 1093–1108. https://doi.org/10.1002/lno.10756

Fréon, P., Cury, P., Shannon, L., & Roy, C. (2005). Sustainable exploitation of small pelagic fish stocks challenged by environmental and ecosystem changes: A review. *BULLETIN OF MARINE SCIENCE*, *76*, 385–462.

Friedenberg, L. E., Bollens, S. M., & Rollwagen-Bollens, G. (2012). Feeding dynamics of larval Pacific herring (*Clupea pallasi*) on natural prey assemblages: The importance of protists. *Fisheries Oceanography*, *21*(2–3), 95–108. https://doi.org/10.1111/j.1365-2419.2011.00611.x

Galasso, C., Celentano, S., Costantini, M., D'Aniello, S., Ianora, A., Sansone, C., & Romano, G. (2020). Diatom-derived polyunsaturated aldehydes activate similar cell death genes in two different systems: Sea urchin embryos and human cells. *International Journal of Molecular Sciences*, *21*(15), Article 15. https://doi.org/10.3390/ijms21155201

Gleason, T. R., & Bengtson, D. A. (1996). Growth, survival and size-selective predation mortality of larval and juvenile inland silversides, *Menidia beryllina* (Pisces; *Atherinidae*). *Journal of Experimental Marine Biology and Ecology*, *199*(2), 165–177. https://doi.org/10.1016/0022-0981(95)00194-8

Hansen, E. (2004). The poly-unsaturated aldehyde 2-trans-4-trans-Decadienal disturbs DNA replication and mitotic events in early sea urchin embryos. *Toxicological Sciences*, *81*(1), 190–197. https://doi.org/10.1093/toxsci/kfh192

Hare, J. A., & Cowen, R. K. (1997). Size, Growth, Development, and Survival of the Planktonic Larvae of *Pomatomus Saltatrix* (pisces: *Pomatomidae*). *Ecology*, *78*(8), 2415–2431. https://doi.org/10.1890/0012-9658(1997)078[2415:SGDASO]2.0.CO;2

He, J.-H., Guo, S.-Y., Zhu, F., Zhu, J.-J., Chen, Y.-X., Huang, C.-J., Gao, J., Dong, Q., Xuan, Y.-X., & Li, C.-Q. (2012). A zebrafish phenotypic assay for assessing drug-induced hepatotoxicity. *Journal of Pharmacological and Toxicological Methods*, *67*. https://doi.org/10.1016/j.vascn.2012.10.003

Hjort, J. (1914). Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. ICES.

Houde, E. D. (2002). Mortality. *Fishery Science: The Unique Contributions of Early Life Stages*.

Houde, E. D. (2008). Emerging from Hjort's Shadow. *Journal of Northwest Atlantic Fishery Science*, *41*, 53–70. https://doi.org/10.2960/J.v41.m634

Houde, E. D., & Schekter, R. C. (1980). Feeding by marine fish larvae: Developmental and functional responses. *Environmental Biology of Fishes*, *5*(4), 315–334. https://doi.org/10.1007/BF00005186

Huriaux, F., Mélot, F., Vandewalle, P., Collin, S., & Focant, B. (1996). Parvalbumin isotypes in white muscle from three teleost fish: Characterization and their expression during development. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *113*(3), 475–484. https://doi.org/10.1016/0305-0491(95)02066-7

Ianora, A., Bentley, M. G., Caldwell, G. S., Casotti, R., Cembella, A. D., Engström-Öst, J., Halsband, C., Sonnenschein, E., Legrand, C., Llewellyn, C. A., Paldavičienë, A., Pilkaityte, R., Pohnert, G., Razinkovas, A., Romano, G., Tillmann, U., & Vaiciute, D. (2011). The relevance of marine chemical ecology to plankton and ecosystem function: An emerging field. *Marine Drugs*, *9*(9), 1625–1648. https://doi.org/10.3390/md9091625

Ianora, A., & Miralto, A. (2010). Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: A review. *Ecotoxicology*, *19*(3), 493–511. https://doi.org/10.1007/s10646-009-0434-y

Ianora, A., Miralto, A., Poulet, S. A., Carotenuto, Y., Buttino, I., Romano, G., Casotti, R., Pohnert, G., Wichard, T., Colucci-D'Amato, L., Terrazzano, G., & Smetacek, V. (2004). Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. *Nature*, *429*(6990), Article 6990. https://doi.org/10.1038/nature02526

Ianora, A., & Poulet, S. A. (1993). Egg viability in the copepod *Temora stylifera*. *Limnology and Oceanography*, *38*(8), 1615–1626. https://doi.org/10.4319/lo.1993.38.8.1615

Ianora, A., Poulet, S. A., Miralto, A., & Grottoli, R. (1996). The diatom *Thalassiosira rotula* affects reproductive success in the copepod *Acartia clausi*. *Marine Biology*, *125*(2), 279.

Illing, B., Moyano, M., Niemax, J., & Peck, M. A. (2015). Direct effects of microalgae and protists on herring (*Clupea harengus*) yolk sac larvae. *PLOS ONE*, *10*(6), e0129344. https://doi.org/10.1371/journal.pone.0129344

Incardona, J., & Scholz, N. (2017). Environmental Pollution and the Fish Heart. In *Fish Physiology*. https://doi.org/10.1016/bs.fp.2017.09.006

Jager, K. J., van Dijk, P. C., Zoccali, C., & Dekker, F. W. (2008). The analysis of survival data: The Kaplan–Meier method. *Kidney International*, *74*(5), 560–565. https://doi.org/10.1038/ki.2008.217

Jahncke, J., Checkley, D. M., & Hunt, G. L. (2004). Trends in carbon flux to seabirds in the Peruvian upwelling system: Effects of wind and fisheries on population regulation. *Fisheries Oceanography*, *13*(3), 208–223. https://doi.org/10.1111/j.1365-2419.2004.00283.x

James Cooper, W., VanHall, R., Sweet, E., Milewski, H., DeLeon, Z., Verderber, A., DeLeon, A., Galindo, D., & Lazono, O. (2020). Functional morphogenesis from embryos to adults: Late development shapes trophic niche in coral reef damselfishes. *Evolution & Development*, *22*(3), 221–240. https://doi.org/10.1111/ede.12321

Jewson, D. H., Lowry, S. F., & Bowen, R. (2006). Co-existence and survival of diatoms on sand grains. *European Journal of Phycology*, *41*(2), 131–146. https://doi.org/10.1080/09670260600652903

Koski, M., Wichard, T., & Jónasdóttir, S. H. (2008). "Good" and "bad" diatoms: Development, growth and juvenile mortality of the copepod *Temora longicornis* on diatom diets. *Marine Biology*, *154*(4), 719–734. https://doi.org/10.jager1007/s00227-008-0965-4

LaMorte, W.W. (2016). Survival Analysis. https://sphweb.bumc.bu.edu/otlt/MPHModules/BS/BS704_Survival/index.html

Lauritano, C., Andersen, J. H., Hansen, E., Albrigtsen, M., Escalera, L., Esposito, F., Helland, K., Hanssen, K. Ø., Romano, G., & Ianora, A. (2016). Bioactivity screening of microalgae for antioxidant, anti-Inflammatory, anticancer, anti-Diabetes, and antibacterial Activities. *Frontiers in Marine Science*, *3*. https://www.frontiersin.org/articles/10.3389/fmars.2016.00068

Lebour, M. V. (1924). The Food of Young Herring. *Journal of the Marine Biological Association of the United Kingdom*, *13*(2), 325–330. https://doi.org/10.1017/S0025315400007542

Lettieri, A., Esposito, R., Ianora, A., & Spagnuolo, A. (2015). *Ciona intestinalis* as a marine model system to study some key developmental genes targeted by the diatom-derived aldehyde decadienal. *Marine Drugs*, *13*(3), 1451–1465. https://doi.org/10.3390/md13031451

Linnaeus, C. (1789). *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species; cum characteribus, differentiis, synonymis, locis*. apud J.B. Delamolliere.

Linné, C. von, & Gesellschaft, D. Z. (n.d.). *Caroli Linnæi Systema naturæ* (Editio decima, 1758, / cura Societatis Zoologicae Germanicae iterum edita, pp. 1–842). Sumptibus Guilielmi Engelmann. https://www.biodiversitylibrary.org/item/80764

Logan, J. M., Rodríguez-Marín, E., Goñi, N., Barreiro, S., Arrizabalaga, H., Golet, W., & Lutcavage, M. (2011). Diet of young Atlantic bluefin tuna (*Thunnus thynnus*) in eastern and western Atlantic foraging grounds. *Marine Biology*, *158*(1), 73–85. https://doi.org/10.1007/s00227-010-1543-0

Loosanoff, V. L. (1938). The Spawning run of the pacific surf smelt, *Hypomesus pretiosus* [Girard]. *Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie*, *36*(1), 170–183. https://doi.org/10.1002/iroh.19380360106

Magnussen, E. (2011). Food and feeding habits of cod (*Gadus morhua*) on the Faroe Bank. *ICES Journal of Marine Science*, *68*(9), 1909–1917. https://doi.org/10.1093/icesjms/fsr104

Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., Wincker, P., Iudicone, D., de Vargas, C., Bittner, L., Zingone, A., & Bowler, C. (2016). Insights into global diatom distribution and diversity in the world's ocean. *Proceedings of the National Academy of Sciences*, *113*(11), E1516–E1525. https://doi.org/10.1073/pnas.1509523113

Martínez, K. A., Lauritano, C., Romano, G., & Ianora, A. (2018). Marine microalgae with anti-cancer properties. *Marine Drugs*, *16*. https://doi.org/10.3390/md16050165

Miller, T. J., Crowder, L. B., Rice, J. A., & Marschall, E. A. (1988). Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*(9), 1657–1670. https://doi.org/10.1139/f88-197

Miralto, A., Barone, G., Romano, G., Poulet, S. A., Ianora, A., Russo, G. L., Buttino, I., Mazzarella, G., Laabir, M., Cabrini, M., & Giacobbe, M. G. (1999). The insidious effect of diatoms on copepod reproduction. *Nature*, *402*(6758), Article 6758. https://doi.org/10.1038/46023

Nayeem, M. A. (2018). Role of oxylipins in cardiovascular diseases. *Acta Pharmacologica Sinica*, *39*(7), Article 7. https://doi.org/10.1038/aps.2018.24

Palmer, E., Tushingham, S., & Kemp, B. M. (2018). Human use of small forage fish: Improved ancient DNA species identification techniques reveal long term record of sustainable mass harvesting of smelt fishery in the northeast Pacific Rim. *Journal of Archaeological Science*, *99*, 143–152. https://doi.org/10.1016/j.jas.2018.09.014

Paradis, A. R., Pepin, P., & Brown, J. A. (1996). Vulnerability of fish eggs and larvae to predation: Review of the influence of the relative size of prey and predator. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*, 1226–1235. https://doi.org/10.1139/cjfas-53-6-1226

Pauly, D. (1998). Diet composition and trophic levels of marine mammals. *ICES Journal of Marine Science*, *55*(3), 467–481. https://doi.org/10.1006/jmsc.1997.0280

Penttila, D. (2007). *Marine forage fishes in Puget Sound:* Defense Technical Information Center. https://doi.org/10.21236/ADA478081

Peterson, I., & Wroblewski, J. (2011). Mortality rate of fishes in the pelagic ecosystem. *Canadian Journal of Fisheries and Aquatic Sciences*, *41*, 1117–1120. https://doi.org/10.1139/f84-131

Pezzolesi, L., Pichierri, S., Samorì, C., Totti, C., & Pistocchi, R. (2017). PUFAs and PUAs production in three benthic diatoms from the northern Adriatic Sea. *Phytochemistry*, *142*, 85–91. https://doi.org/10.1016/j.phytochem.2017.06.018

Pohnert, G. (2005). Diatom/copepod interactions in plankton: The indirect chemical defense of unicellular algae. *ChemBioChem*, *6*(6), 946–959. https://doi.org/10.1002/cbic.200400348

Prazukin, A. V., Lee, R. I., Firsov, Y. K., & Kapranov, S. V. (2022). Vertical distribution of epiphytic diatoms in relation to the eelgrass *Zostera noltii* canopy biomass and height. *Aquatic Botany*, *176*, 103466. https://doi.org/10.1016/j.aquabot.2021.103466

Prost, I., Dhondt, S., Rothe, G., Vicente, J., Rodriguez, M. J., Kift, N., Carbonne, F., Griffiths, G., Esquerré-Tugayé, M.-T., Rosahl, S., Castresana, C., Hamberg, M., & Fournier, J. (2005). Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. *Plant Physiology*, *139*(4), 1902–1913. https://doi.org/10.1104/pp.105.066274

Quinn, T., Krueger, K., Pierce, K., Penttila, D., Perry, K., Hicks, T., & Lowry, D. (2012). Patterns of Surf Smelt, *Hypomesus pretiosus*, Intertidal spawning habitat use in Puget Sound, Washington State. *Estuaries and Coasts*, *35*(5), 1214–1228. https://doi.org/10.1007/s12237-012-9511-1

Raventos, N., & Macpherson, E. (2005). Effect of pelagic larval growth and size-at-hatching on post-settlement survivorship in two temperate labrid fish of the genus *Symphodus*. *Marine Ecology Progress Series*, *285*, 205–211. https://doi.org/10.3354/meps285205

Ribalet, F., Bastianini, M., Vidoudez, C., Acri, F., Berges, J., Ianora, A., Miralto, A., Pohnert, G., Romano, G., Wichard, T., & Casotti, R. (2014). Phytoplankton cell lysis associated with polyunsaturated aldehyde release in the Northern Adriatic Sea. *PLOS ONE*, *9*(1), e85947. https://doi.org/10.1371/journal.pone.0085947

Ribalet, F., Berges, J. A., Ianora, A., & Casotti, R. (2007a). Growth inhibition of cultured marine phytoplankton by toxic algal-derived polyunsaturated aldehydes. *Aquatic Toxicology*, *85*(3), 219–227. https://doi.org/10.1016/j.aquatox.2007.09.006

Ribalet, F., Wichard, T., Pohnert, G., Ianora, A., Miralto, A., & Casotti, R. (2007b). Age and nutrient limitation enhance polyunsaturated aldehyde production in marine diatoms. *Phytochemistry*, *68*(15), 2059–2067. https://doi.org/10.1016/j.phytochem.2007.05.012

Risso, A. (1810). *Ichthyologie de Nice, ou, Histoire naturelle des poissons du département des Alpes Maritimes* (pp. 1–454). F. Schoell. https://www.biodiversitylibrary.org/item/30472

Romano, G., Miralto, A., & Ianora, A. (2010). Teratogenic effects of diatom metabolites on sea urchin *Paracentrotus lividus* embryos. *Marine Drugs*, *8*(4), 950–967. https://doi.org/10.3390/md8040950

Romano, G., Russo, G. L., Buttino, I., Ianora, A., & Miralto, A. (2003). A marine diatom-derived aldehyde induces apoptosis in copepod and sea urchin embryos. *Journal of Experimental Biology*, *206*(19), 3487–3494. https://doi.org/10.1242/jeb.00580

Ruocco, N., Albarano, L., Esposito, R., Zupo, V., Costantini, M., & Ianora, A. (2020). Multiple roles of diatom-derived oxylipins within marine environments and their potential biotechnological applications. *Marine Drugs*, *18*(7), Article 7. https://doi.org/10.3390/md18070342

Ruocco, N., Annunziata, C., Ianora, A., Libralato, G., Manfra, L., Costantini, S., & Costantini, M. (2019). Toxicity of diatom-derived polyunsaturated aldehyde mixtures on sea urchin *Paracentrotus lividus* development. *Scientific Reports*, *9*(1), 517. https://doi.org/10.1038/s41598-018-37546-y

Sansone, C., Braca, A., Ercolesi, E., Romano, G., Palumbo, A., Casotti, R., Francone, M., & Ianora, A. (2014). Diatom-derived polyunsaturated aldehydes activate cell death in human cancer cell lines but not normal cells. *PLOS ONE*, *9*(7), e101220. https://doi.org/10.1371/journal.pone.0101220

Schmidt-Nielsen, K. (1972). Locomotion: Energy cost of swimming, flying, and running. Science (New York, N.Y.), 177(4045), 222–228. https://doi.org/10.1126/science.177.4045.222

Sigolo, C. A. O., Di Mascio, P., Kowaltowski, A. J., Garcia, C. C. M., & Medeiros, M. H. G. (2008). Trans,trans-2,4-decadienal induces mitochondrial dysfunction and oxidative stress. *Journal of Bioenergetics and Biomembranes*, *40*(2), 103–109. https://doi.org/10.1007/s10863-008-9137-y

Smetacek, V. (1999). Diatoms and the ocean carbon cycle. *Protist*, *150*(1), 25–32. https://doi.org/10.1016/S1434-4610(99)70006-4

Smigielski, A. S., Halavik, T. A., Buckley, L. J., Drew, S. M., & Laurence, G. C. (1984). Spawning, embryo development and growth of the American sand lance *Ammodytes americanus* in the laboratory. *Marine Ecology Progress Series*, *14*(2/3), 287–292.

Spitsbergen, J. M., Walker, M. K., Olson, J. R., & Peterson, R. E. (1991). Pathologic alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo- p-dioxin as. *Aquatic Toxicology*, *19*(1), 41–71. https://doi.org/10.1016/0166-445X(91)90027-7

Spittler, P., Brenning, U., & Arlt, G. (1990). Protozoans—The first food of larval herring (*Clupea harengus L.*) *Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie*, *75*(5), 597–603. https://doi.org/10.1002/iroh.19900750503

Steiner, N. S., Cheung, W. W. L., Cisneros-Montemayor, A. M., Drost, H., Hayashida, H., Hoover, C., Lam, J., Sou, T., Sumaila, U. R., Suprenand, P., Tai, T. C., & VanderZwaag, D. L. (2019). Impacts of the changing ocean-sea ice system on the key forage fish Arctic cod (*Boreogadus Saida*) and subsistence fisheries in the Western Canadian Arctic—evaluating linked climate, ecosystem and economic (CEE) models. *Frontiers in Marine Science*, *6*. https://www.frontiersin.org/articles/10.3389/fmars.2019.00179

Stonik, V., & Stonik, I. (2015). Low-molecular-weight metabolites from diatoms: structures, biological roles and biosynthesis. *Marine Drugs*, *13*(6), 3672–3709. https://doi.org/10.3390/md13063672

Thompson, P. M., Mcconnell, B. J., Tollit, D. J., Mackay, A., Hunter, C., & Racey, P. A. (1996). Comparative distribution, movements and diet of harbour and grey seals from Moray Firth, N. E. Scotland. *Journal of Applied Ecology*, *33*(6), 1572–1584. https://doi.org/10.2307/2404795

Tourdot, B., Ahmed, I., & Holinstat, M. (2014). The emerging role of oxylipins in thrombosis and diabetes. *Frontiers in Pharmacology*, *4*. https://www.frontiersin.org/articles/10.3389/fphar.2013.00176

Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., Bittner, L., Dugdale, R., Finkel, Z., Iudicone, D., Jahn, O., Guidi, L., Lasbleiz, M., Leblanc, K., Levy, M., &

Pondaven, P. (2018). Influence of diatom diversity on the ocean biological carbon pump. *Nature Geoscience*, *11*(1), 27–37. https://doi.org/10.1038/s41561-017-0028-x

Urushibata, H., Sasaki, K., Takahashi, E., Hanada, T., Fujimoto, T., Arai, K., & Yamaha, E. (2021). Control of developmental speed in zebrafish embryos using different incubation temperatures. *Zebrafish*, *18*(5), 316–325. https://doi.org/10.1089/zeb.2021.0022

Van Leeuwen, J. L., Voesenek, C. J., & Müller, U. K. (2015). How body torque and Strouhal number change with swimming speed and developmental stage in larval zebrafish. *Journal of The Royal Society Interface*, *12*(110), 20150479. https://doi.org/10.1098/rsif.2015.0479

Varrella, S., Romano, G., Ianora, A., Bentley, M., Ruocco, N., & Costantini, M. (2014). Molecular response to toxic diatom-derived aldehydes in the sea urchin *Paracentrotus lividus*. *Marine Drugs*, *12*(4), 2089–2113. https://doi.org/10.3390/md12042089

Vidoudez, C., Casotti, R., Bastianini, M., & Pohnert, G. (2011). Quantification of dissolved and particulate polyunsaturated aldehydes in the adriatic Sea. *Marine Drugs*, *9*(4), 500–513. https://doi.org/10.3390/md9040500

Vidoudez, C., & Pohnert, G. (2008). Growth phase-specific release of polyunsaturated aldehydes by the diatom *Skeletonema marinoi*. *Journal of Plankton Research*, *30*(11), 1305–1313. https://doi.org/10.1093/plankt/fbn085

Voesenek, C. J., Muijres, F. T., & van Leeuwen, J. L. (2018). Biomechanics of swimming in developing larval fish. *Journal of Experimental Biology*, *221*(1), jeb149583. https://doi.org/10.1242/jeb.149583

Weise, M., & Harvey, J. (2008). Temporal variability in ocean climate and California sea lion diet and biomass consumption: Implications for fisheries management. *Marine Ecology-Progress Series - MAR ECOL-PROGR SER*, *373*, 157–172. https://doi.org/10.3354/meps07737

Wichard, T., Poulet, S. A., Halsband-Lenk, C., Albaina, A., Harris, R., Liu, D., & Pohnert, G. (2005). Survey of the chemical defense potential of diatoms: screening of fifty species for α,β,γ,δ-unsaturated aldehydes. *Journal of Chemical Ecology*, *31*(4), 949–958. https://doi.org/10.1007/s10886-005-3615-z

Wolfram, S., Nejstgaard, J. C., & Pohnert, G. (2014). Accumulation of polyunsaturated aldehydes in the gonads of the copepod *Acartia tonsa* revealed by tailored fluorescent probes. *PLoS ONE*, *9*(11), e112522. https://doi.org/10.1371/journal.pone.0112522

Yin, M. C., & Blaxter, J. H. S. (1987). Feeding ability and survival during starvation of marine fish larvae reared in the laboratory. *Journal of Experimental Marine Biology and Ecology*, *105*(1), 73–83. https://doi.org/10.1016/S0022-0981(87)80030-8

Yúfera, M., & Darias, M. J. (2007). The onset of exogenous feeding in marine fish larvae. *Aquaculture*, *268*(1), 53–63. https://doi.org/10.1016/j.aquaculture.2007.04.050

Zhou, Y., Zhang, Y., Li, F., Tan, L., & Wang, J. (2017). Nutrients structure changes impact the competition and succession between diatom and dinoflagellate in the East China Sea. *Science of The Total Environment*, *574*, 499–508. https://doi.org/10.1016/j.scitotenv.2016.09.092