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Ranked effects of heavy metals on marine bivalves in laboratory mesocosms: A meta-analysis

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ABSTRACT

Bivalves are commonly used as biomonitors for heavy metal pollution in marine environments because they accumulate heavy metal ions quickly, are sessile, abundant, and widely dispersed, and adult mortality from contamination is rare. However, the breadth of experiments used to measure the effect of heavy metal contamination can obscure general trends. It is unclear which heavy metals cause the most severe effects, how severity varies with exposure concentration and duration, and whether effects vary with level of biological organization. I conducted a meta-analysis of 48 mesocosm studies on the effects of heavy metal ions – silver, cadmium, copper, mercury, lead, and zinc – on marine bivalves. The ordering of effect sizes was Pb > Hg > Cu > Zn > Cd > Ag. The significance and direction of concentration and duration as moderators depended on the metal and the biological level. Future studies should consider non-linear effects over time and concentration, and measure both bioaccumulation and effect of the metals being studies.

1. Introduction

The use of bivalves to monitor aquatic pollution, particularly for heavy metal contamination in marine environments, has been common since the 1970s (Goldberg, 1975). Many studies have established that heavy metals have significant effects on bivalves in terms of, for example, genetic diversity (Breitwieser et al., 2016), tissue and cell necrosis (Sheir and Handy, 2010), immune system health (Ivanina et al., 2016), reproductive health (Liu et al., 2014), and filtration rate (Sobrino-Figueroa and Cáceres-Martinez, 2014). Nonetheless, information regarding the conservation status of marine bivalves is scarce. A search of "bivalve" in the IUCN Red List of Threatened Species, the most exhaustive global database of species' conservation status, returns a list of only ten species all of which are freshwater dwelling.

Because an organism's response to contaminants must be known in order for it to serve as a functional biomonitor, numerous studies have examined the effects of heavy metal contamination on bivalves. The response of bivalve species to heavy metal contamination is complex. Each species responds differently to different metals, even when other biotic and abiotic conditions are equal (Vijayavel et al., 2007). While many metals are toxic in large doses, some heavy metals such as Fe and Cu are micronutrients, and can therefore have a positive effect at low concentration (Yeung et al., 2016). The rate of accumulation of metals in tissues depends on biotic and abiotic factors, such as water salinity (Gamain et al., 2016) and temperature (Boukadida et al., 2016), which vary seasonally and geographically (Phillips, 1980). These factors can influence the relative toxicity of the metal, in addition to the rate of accumulation. Interaction effects have also been observed: the toxicity of a metal can be increased or decreased by the presence of other heavy metals or contaminants (Fathallah et al., 2013). Finally, metal toxicity often exhibits a non-linear dose dependency (Amachree et al., 2013).

Understanding the effects of heavy metal contamination on marine bivalves is further complicated by the fact that the effects are rarely fatal in adult bivalves, so experiments do not have unambiguous endpoints. While mortality is a common measure for larvae or embryos (for example, Gamain et al., 2016; Fathallah et al., 2013), the majority of experiments performed on adult or juvenile bivalves measure morbidity instead. Low mortality rates from heavy metal contamination are useful in biomonitoring studies because they ensure the organism can be used over a long period of time or in highly contaminated environments. However, the diversity of measures of morbidity – such as lowered filtration rate or increased oxidative stress markers – makes quantitative comparison among studies challenging.

Meta-analysis is a promising means of extracting information on overarching trends from biomonitoring studies, which have a wide range of study sizes, methods, and metrics for measuring biological effects. Meta-analysis is a statistical synthesis that combines and weights published results within a defined group of studies in order to establish a weighted average effect – the "effect size". It has already

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Table 1
Summary of toxicity, uses, chronology, human impacts, and meta-analytic effect sizes of heavy metals in marine bivalves. Geography-specific information, such as product bans, is for USA (Wang et al., 2009; Banfálvi, 2011).

Metal	Mechanism of toxicity	Industrial uses	Time period of use	Impact on humans	Effect size
Ag	Interferes with sodium-potassium ATPase; low continuous exposure may harm reproduction	Byproduct of Au, Pb, Zn, Cu refining; used in photography and photovoltaic panels	Photography use declining since 1999.	Silver salts toxic to humans; other forms non-toxic.	1.61
Cd	Catalyzes formation of ROS, depletes antioxidants.	Batteries, industrial paints, electroplating, plastics	Still in use but heavily monitored.	Highly toxic in low doses.	1.67
Cu	Impairs enzymes, can cause oxidative stress & Zn deficiency.	Anti-fouling agent on boat hulls (replaced tributyltin)	Banned in 2011 in Washington State for recreational boats.	MCL in water is 1.3 mg/l (EPA)	2.12
Hg	Inhibits selenoenzymes (ex. thioredoxin reductase), increasing oxidative damage	Felting of fur for hats (obsolete), deep earth mining waste product, manufacture of chlorine/caustic soda, electrodes, cosmetics, thermometers, lighting.	Industrial production chlorine/caustic soda phased out 1985. USA: non- prescription Hg fever thermometers banned 2003.	Very toxic: can result in death. Bioaccumulation in fish can be mechanism of poisoning in humans.	2.35
Pb	Reactive radicals damage DNA, cell membrane; interference w/ enzymes; inflammatory protein production	Obsolete: household items, plumbing, leaded paint (inc. marine paint), anti-noc	Anti-knock: 1921–1986 Leaded paint: banned 2000	Highly toxic and highly regulated. Though banned, products that contain lead (ex. paint) are still in use.	4.34
Zn	Suppression Cu and Fe absorption; Zn salts corrode tissue	Batteries, steel/iron coating, metal alloys, roofing, print processes	N/A	Low toxicity in humans	1.82

Table 2
Summary of the random-effects results. p-Values: ***0.001; **0.01; *0.05. none = not significant. The categories in bold have only two studies. "N/A" signifies a single study in the category: effect sizes are not shown for these because the results are not meaningful. While Hg and Pb had the largest effect size, they also had the largest standard error. Cd and Cu, which had the largest number of data points, had some of the smallest standard errors. Interestingly, Ag also had a very small standard error despite not having as many data points (Fig. 2).

Metal	Effect size (SE)	Category	Effect size (SE)
Ag	0.207 (0.036)***	Cellular	0.141 (0.034)***
		Physiological	**0.344 (0.13)***
		Population	N/A
Cd	0.222 (0.026)***	Cellular	0.203 (0.019)***
		Physiological	0.749 (0.090)***
		Population	0.059 (0.014)***
Cu	0.326 (0.040)***	Cellular	0.124 (0.025)***
		Physiological	0.130 (0.018)***
		Population	0.480 (0.074)***
Hg	0.372 (0.12)**	Cellular	0.0904 (0.043)*
		Physiological	0.191 (0.078)*
		Population	0.537 (0.24)*
Pb	0.638 (0.12)***	Cellular	0.242 (0.031)***
		Physiological	**1.35 (0.27)*****
		Population	1.71 (0.47)**
Zn	0.259 (0.067)***	Cellular	**0.019 (0.0076)***
		Physiological	0.517 (0.14)***
		Population	0.320 (0.10)**

proven useful in other highly empirical disciplines, such as such as education (Hedges et al., 1994), ecology (Osenberg et al., 1999), and medicine (Sutton et al., 2000). However, the only formal meta-analysis of heavy metal effects on marine organisms is O'Brien and Keough's (2014) study, which focused on the effects of a single metal (copper) on marine invertebrates.

Here, I use meta-analysis of bivalve heavy metal contamination literature to differentiate the effects of six different heavy metals: silver (Ag), cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn). These metals were chosen based on the number of papers available and their known effects on living organisms (Table 1). Because bivalve responses are increased morbidity (diminished functionality) rather than increased mortality (decreased survival), and because measures of morbidity are highly diverse, this meta-analysis uses dimensionless effect-size measurements. It cannot quantify the severity of an effect in an absolute sense, but can rank effects among contaminants. (See Table 2.)

My hypotheses were that lead and mercury would have the largest effect sizes, as their severely deleterious effect on biological tissue has been quantified in many species, including humans (Papanikolaou et al., 2005; Zahir et al., 2005) and several species of plants (Verma and Dubey, 2003; Patra and Sharma, 2000). I also expected that non-essential metals (i.e. ones that are not required in trace amounts for essential biochemical and physiological processes in metabolism, reproduction, or growth) would have more deleterious effects, because they are more likely to cause damage even at low concentrations: of the metals listed above, Pb, Cd, Ag and Hg are in this category (Tchounwou et al., 2012). I expected effect size to decline from cellular to physiological to population, because damage at lower orders of biological organization is only likely to "scale up" and impede functionality at higher orders within an individual above some minimal level of damage. For example, low levels of lysosomal damage, a cellular level effect, may not cause measurable damage at physiological or population levels unless the damage reaches a certain level of severity. While biomagnification up through trophic levels can and does occur for some heavy metals – for instance Hg – this upward transfer is much weaker when the highest level of organization is a population rather than, for example, a community or entire ecosystem (van der Velden et al., 2013). Lastly, I expected duration of exposure to be a stronger moderator of effect than exposure concentration, because several studies showed nonlinear or inconsistent changes with concentration (for example, Amachree et al., 2013; Sobrino-Figueroa and Cáceres-Martinez, 2014).

2. Methods

2.1. Gathering studies

Studies were gathered through a comprehensive search of Google Scholar, ScienceDirect, and the University of Chicago's ArticlePlus. The initial search allowed me to narrow heavy metal contamination down to six metals of interest: Cu, Cd, Hg, Ag, Zn and Pb. These were featured most extensively in papers and therefore had the highest likelihood of providing a sufficient amount of data for robust results. These metals are all a) prominent marine pollutants, or b) known to have severe potential effects on biological tissue, and were chosen based on their actual/potential effects (see Table 1 for more information on each metal).

Several criteria determined whether or not a study was included in the meta-analysis:

1. The study was on marine bivalves.
2. The study measured one or more biological effects of heavy metal accumulation, not just the rate of metal accumulation in tissues.
3. The study was conducted in a laboratory.
4. The report included the following information: sample size, effect size and variance for control and experimental trials of known variance type (i.e. it was stated whether standard error or standard deviation was used), aquatic concentration of the heavy metal, and duration of the experiment.
5. The heavy metal was added to the mesocosm in ionic form.

A total of 46 studies met these criteria, yielding 812 data points for biologic effects (Fig. 1). Each data point represented the endpoint of a particular experiment. For example, the effect of 100 μg/l of copper on the dry tissue weight of *Mytilus edulis* would be one data point. Studies (published papers) contained multiple data points when they tested multiple metals, species, concentrations, or experiments. Studies only contained one data point if they tested these over a period of time (for example, if a study tested the effect of some concentration of metal after 3, 7, and 21 days, only the 21st day was included). An experiment is operationally defined as a scientific test that measures some response variable. The amount of metal accumulated by an individual does not count as a response variable because it does not indicate any strain as a result of stress from contamination.

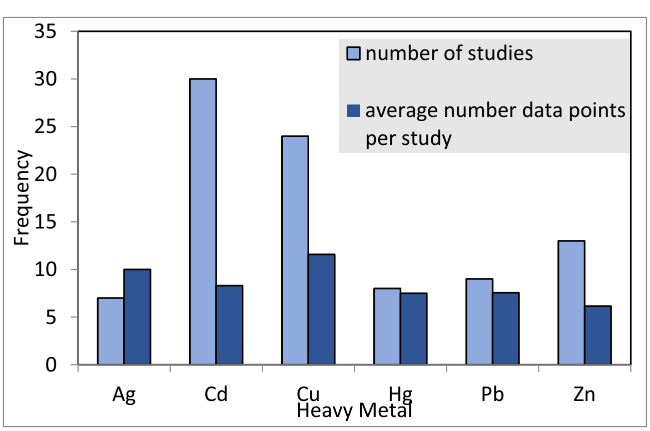

Fig. 1. Distribution of data points and studies by metal. On average, each study contained about 10 data points. Cadmium and copper were the most frequently tested metals, despite not being the most toxic for bivalves.

For each data point I recorded information on: study name, author, year, and journal; heavy metal and its concentration; bivalve species; experimental duration; experiments measuring a response variable (i.e. filtration rate); effect size and variance of control and experimental trials. After this information was acquired from the studies, it became apparent that most experiments could be grouped into three levels of biological organization: cellular, physiological, and population level effects. Physiological level effects measure at the level of the individual, organ, or tissue, and population level effects measure mortality or reproduction. This is similar to the levels constructed in a meta-analysis by O'Brien and Keough (2014) although theirs focused on higher levels of organization (individual, population, and community). A comprehensive list of the response variables included in each category is available in Table 5B.

2.2. Statistical analysis

Studies were analyzed in excel and in R using the package "metafor". In excel, the data was transformed to measure the absolute value of the log of effect size:

$$= \left| log\,\frac{experimental\ effect\ size}{control\ effect\ size} \right|$$

Variance was calculated according to Hedges et al., 1999:

$$\frac{(SD_e)^2}{n_e X_e} + \frac{(SD_c)^2}{n_c X_c}$$

where SD is the standard deviation, n is the sample size, X is the mean, and e and c are experimental and control groups. Although Hedges' paper did not use absolute value, this was necessary due to the nature of the data. Whether the experimental effect was greater than or less than the control effect was not a necessary indicator of the effect being negative or positive. For example, a decreased filtration rate indicates a negative effect, but an *increased* rate of larval abnormalities indicates a negative effect also. To accurately record true positive/negative results, I took the absolute value of all data points then manually added a minus sign to data points that actually demonstrated a positive effect. A positive value therefore implies a negative effect. To eliminate statistical dependence, all but the final point of studies that took measurements over a length of time were eliminated, whether the study took measurements with or without replacement. The final point did not always show the largest effect.

A nested study model was used to compare and contrast effect size. I used a random effects model, which, unlike a fixed effects model, does not assume all variation between studies is due to sampling error. This also makes results more generalizable (Borenstein et al., 2009). Subsets of the data were created for type of heavy metal and level of biological response. Effect sizes for each subset were calculated separately (e.g., subset = "Cu" or subset = "population" level response) and then together (e.g., subset = "Cu" *and* "population" level response). Finally, for a mixed effect model, which allows the inclusion of moderators, the metal subsets were reanalyzed with concentration and duration as numerical moderators, and with level of biological organization as a categorical moderator.

3. Results

3.1. Variation among metals

Of the six metals measured, the order of effect size was Pb > Hg > Cu > Zn > Cd > Ag. Values can be compared from the second column of Table 3 under the heading "Effect size". The results indicate that Pb and Hg are the most toxic, as hypothesized. However, the division between essential and nonessential metals is not a strong predictor for toxicity: Cu and Zn are essential metals, while Cd, Ag, Hg,

Table 3
Summary of results for numerical and categorical moderators. For numerical moderators, the results signify that an increase or decrease of one unit of the moderator corresponds to the listed increase or decrease in the log of effect size. For categorical moderators, the results test the null hypothesis that effect size for a biological level is not significantly different from effect size overall. They cannot be used to conclude that a level is significantly different from any other level because the test does not explicitly compare them to each other. For comparison between biological levels, see Fig. 3. Significance codes: ***0.001; **0.01; *0.05; none = not significant.

Metal	Random effects result (SE)	Concentration (SE)	Duration (SE)	Category: cellular (SE)	Category: physiological (SE)	Category: population (SE)
Ag	0.207 (0.036)	0.0003 (0.0006)	−0.0034 (0.0076)	0.136 (0.12)	0.407 (0.16)*	0.320 (0.071)***
Cd	0.222 (0.026)	0.00 (0.00)***	0.0059 (0.0026)*	0.0643 (0.068)	0.239 (0.061)***	0.038 (0.039)
Cu	0.191 (0.078)	0.0003 (0.0002)	−0.0007 (0.002)	0.168 (0.091)	0.154 (0.13)	0.458 (0.054)***
Hg	0.372 (0.12)	−0.0019 (0.002)	−0.0113 (0.028)	0.507 (0.43)	0.394 (0.57)	0.665 (0.22)**
Pb	0.638 (0.12)	0.0013 (0.0002)***	0.0159 (0.0067)*	−0.758 (0.26)*	0.196 (0.20)	0.0086 (0.17)
Zn	0.259 (0.067)	0.0001 (0.0001)	−0.0094 (0.0075)	0.110 (0.21)	0.226 (0.20)	0.327 (0.092)***
All Metals	0.308 (0.023)	0.00 (0.00)***	−0.0014 (0.0013)	0.197 (0.0450)***	0.280 (0.40)***	0.40 (0.034)***

and Pb are not. Essential metals are not less toxic than nonessential metals, contrary to my hypothesis. Recall that, for all results, a more positive value indicates a more negative effect on bivalves.

3.2. Variation among levels

Analyzing effects by level of biological organization, the order of effect size was cellular < physiological < population (with effect sizes and standard errors of 0.151*** (0.012), 0.314*** (0.035), 0.413*** (0.046) respectively). Effect size increases with the level of organization, which is the opposite of my hypothesis. *** indicates a p-value of 0.001, indicating the difference was significant.

3.3. Numerical moderators

In the mixed-effects model, concentration was a significant moderating factor for Cd, Pb, and all metals combined, but not for Ag, Cu, or Hg (Table 3). Duration was a significant moderator only for Cd and Hg. The moderators were less frequently significant than expected, indicating that heavy metal contamination in bivalves may not have strong time- or dose- response relationships.

3.4. Categorical moderators

The results for categorical moderators were irregular (Table 3, Fig. 3). For Cu and metals overall, each biological level was significantly different from the others. For Zn, Hg, and Ag, population differed significantly from cellular and physiological, but these latter two were not significantly different from each other. For Cd only physiological was significantly different from other biological levels.

Fig. 3 shows the results for testing the differences in effect size between biological levels. The difference between levels was not significant for Pb, and for Cd only the physiological moderator was significantly different from cellular and population levels. For Zn and Hg, moderators overall showed no significant differences (p-values of 0.260 and 0.606 respectively). This means that all of the moderators combined did not significantly change the effect size. The other metals had p-values < 0.05 for the moderators overall. The significance of levels of biological organization on the effects of metals thus depends on the metal in question. Nonetheless, population-level effects did broadly tend to be larger than cellular or physiological level effects (Table 3).

4. Discussion

4.1. Caveats: skewed (non-normal) data

There were two issues with the results: skewed (non-normal) frequency distributions, and unexplained heterogeneity. Skewed frequency distributions are problematic for two reasons. Firstly, both random and mixed effects models for meta-analysis assume that the distribution of the means of studies used in the meta-analysis is normal. Non-normality of data – i.e., a wider spread of values on one side of the mean than the other – indicates that variation between studies is caused by more than just random measurement error, i.e. that there is bias in the data. For tests conducted on the "population" subset of data, this was generally not an issue due to the central limit theorem, which states that means will be approximately normal for sufficiently large data sets (the most common standard for "large dataset" is n > 30) (Higgins et al., 2008). "n" refers to the number of samples tested. Population-level tests, which often analyzed larvae and therefore had very large sample sizes, almost always had an n > 30. However, for physiological and cellular level tests, n was often well below 30 and the n for many data points was around 5, which is far too low to meet the standards of the central limit theorem.

One method to quantify and correct for skew is the trim-and-fill function in the R package metafor. This calculates the number of studies "missing" on one side of the mean, assuming a normal distribution, and then fills in those points and recalculates the effect. It can only be used on random-effects calculations. Analysis shows that about half (16) of the 27 data subsets were skewed, and about three quarters (11) of the skewed data sets were right-skewed, i.e. had a long tail of data points having large effect sizes (Table 4). Recall that each data point does not represent a complete study.

The trim-and-fill results can signal several different kinds of biases acting upon the data. For example, left skew in meta-analyses (a deficit of studies finding small effects) is often the result of the "file cabinet effect", which occurs when studies with neutral or counterintuitive results aren't published. Such omissions are a common issue (Rosenberg, 2005). Right skew is more unusual: it suggests that studies finding stronger results have been omitted, and thus that the overall meta-analytic effect size is probably underestimated. This bias is surprising, given that studies with strong results for negative impacts of metals are more likely to be successfully published.

A closer examination of the studies underlying these patterns within the trim and fill results illuminates a potential explanation. The effect sizes for a metal including all levels of biological organization, or for a level of biological organization including all metals, generally had less skew. This pattern suggests that skew is the result of an insufficient number of data points being available in some data subsets. This is a good explanation because such bias could cause either a right or a left skew. The explanation of insufficient data points in some subsets may also clarify the most curious result of the trim-and-fill results: although the data for the cellular and population levels in the "all metals" category are normal (as would be expected if bias is due to random inadequate sampling, as these subsets have more data points), approximately 50 data points are "missing" from the right side of the physiological level for "all metals". The number of data points in the physiological category (175) is drastically lower than for either cellular or population level effects, which have 317 and 316 data points respectively.

Table 4
The number of missing data points per data subset, and the trim-and-fill recalculation compared to the non-modified result. R = points missing from right side (right skew), L = points missing from left side. SE = standard error.

Data subset	No. missing points	Random effects result (SE)	Trim-and-fill modified result (SE)
Ag cellular	0	0.141 (0.034)	0.141 (0.034)
Ag physiological	3R	0.344 (0.13)	0.506 (0.15)
Ag population	1R	0.311 (0.096)	0.343 (0.093)
Ag all	16R	0.207 (0.036)	0.296 (0.037)
Cd cellular	7R	0.203 (0.019)	0.206 (0.035)
Cd physiological	19R	0.750 (0.090)	0.941 (0.097)
Cd population	21 L	0.059 (0.014)	0.037 (0.017)
Cd all	0	0.222 (0.026)	0.222 (0.026)
Cu cellular	0	0.124 (0.025)	0.124 (0.025)
Cu physiological	0	0.130 (0.018)	0.130 (0.018)
Cu population	0	0.480 (0.074)	0.480 (0.074)
Cu all	0	0.326 (0.040)	0.326 (0.040)
Hg cellular	11 L	0.090 (0.043)	0.0313 (0.027)
Hg physiological	0	0.191 (0.078)	0.191 (0.079)
Hg population	8R	0.537 (0.24)	0.803 (0.20)
Hg all	22R	0.372 (0.12)	0.622 (0.10)
Pb cellular	2 L	0.242 (0.031)	0.236 (0.07)
Pb physiological	0	1.35 (0.27)	1.35 (0.27)
Pb population	10R	1.71 (0.47)	2.46 (0.42)
Pb all	27R	0.638 (0.12)	0.973 (0.12)
Zn cellular	8 L	0.0188 (0.0076)	0.0046 (0.068)
Zn physiological	0	0.517 (0.14)	0.517 (0.14)
Zn population	17R	0.320 (0.10)	0.536 (0.096)
Zn all	0	0.251 (0.067)	0.259 (0.067)
Cellular all	0	0.151 (0.012)	0.151 (0.012)
Physiological all	50R	0.287 (0.036)	0.406 (0.039)
Population all	0	0.413 (0.046)	0.413 (0.046)

Although insufficient data is a compelling argument, it may not be the only factor causing skew. Cu and Cd had the largest number of data points (276 and 248, respectively) of the metal subsets, but Cu exhibits no bias in any level and all of the subsets of cadmium are biased, without a clear pattern (7R, 19R, and 21 L for cellular, physiological, and population level results, respectively). Cd had more studies than Cu despite similar numbers of data points, so statistical dependence from taking multiple data points from a single study cannot explain this effect. It is possible that, for some reason, cadmium requires a larger number of studies than copper.

Unfortunately, while under-sampling almost definitely influenced results, it is not an easily resolvable error. I searched through hundreds of studies over several databases over many months, and the 48 studies used in this meta-analysis are an exhaustive set that fit all the requirements listed in the *Methods* section (barring searching in foreign languages). An ability to comprehend the methods and discussion sections is essential for inclusion in the meta-analysis: data-mining of numerical tables does not suffice, which is why foreign-language studies were not included.

4.2. Caveats: unexplained heterogeneity

Another issue encountered in this study is that a large amount of heterogeneity (variability) in the data is unexplained, either by the model or by any moderator. High study heterogeneity, as measured by I^2 – ranging from 9.0% for the mercury/cellular subset to 99.6% for the copper/population subset in the mixed-effects model, with an average of 63.8% – was present across all data subsets. I^2 measures the percent of variance between studies that isn't explained by the model or moderators. I^2 values can reflect the size of the subset as well as unaccounted variability. Some unexplained variation is expected given the diverse studies included in the meta-analysis, and many important moderators might exist other than the ones included. Unexamined potential sources of heterogeneity include: bivalve species used in the experiment, location where experiment was performed or where specimen originated from, date of study, and experimental set up (such as food source, rate of feeding, water temperature, and salinity or other abiotic factors of the ambient environment). Unfortunately, not all of these sources of heterogeneity are consistently documented in mesocosm studies, and so it would be very difficult to obtain a definitive answer as to whether these are the cause of the unexplained heterogeneity.

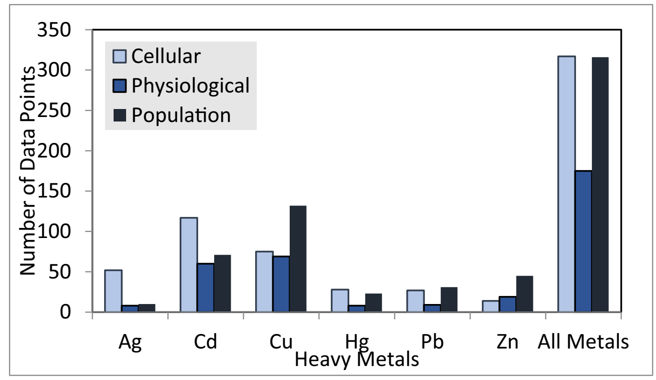

Fig. 2. Distribution of data points by level of biological organization. Overall, twice as many data points were generated by studies of effects at the cellular and population levels than at the physiological level.

4.3. Variation among metals

The finding that Pb and Hg have the highest effect sizes is not surprising. These elements are widely acknowledged as some of the most damaging heavy metals to biological tissues, and this may be why they are under-represented in the studies compiled here (Fig. 2). In studies that measured multiple metals (for example, Sobrino-Figueroa and Cáceres-Martinez, 2014; Fathallah et al., 2013) these two metals consistently had the largest effect.

Cu and Cd were the most widely studied elements. The finding that Zn has more deleterious effects that Cd is therefore somewhat surprising, given that it receives less experimental attention. This implies that the effects of Zn in marine systems may be underestimated as well as understudied, perhaps due to its low impact on human health (Plum et al., 2010).

4.4. Variation among levels of biological organization

Levels of biological organization were tested as data subsets and as categorical moderators. For data subsets, the order of effect sizes among levels – cellular < physiological < population – is unexpected because organisms generally have to experience deleterious effects at the cellular or physiological level before the population level. For example, I expected that an organism would have to experience fairly high levels of oxidative damage at the cellular level before the effects of this damage exceeded the limits of detection at the physiological damage, and of course that physiological damage would have to be high in order for population-level effects to occur. The unexpectedly high ranking of effects at the population level can be explained by the fact that the effects of heavy metals on the most sensitive life stages for bivalves, the embryo and larval stages, are generally only reported at the population

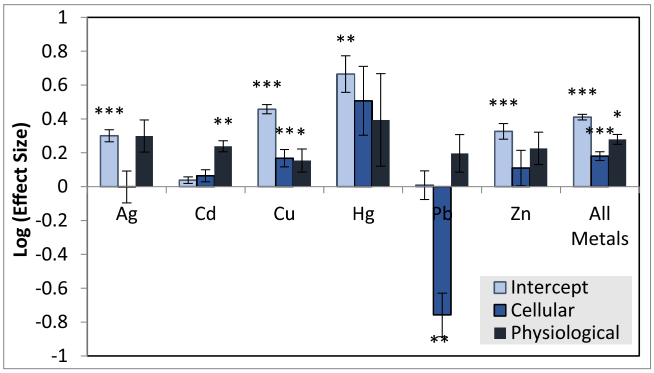

Fig. 3. Comparison of categorical moderators. The type of mixed effects analysis displayed in this graph estimates the difference *between* categories. An value significantly different from zero indicates that there is a difference in the log of effect size between categories. For Zn and Hg only, the moderators overall were not significant (p-values of 0.260 and 0.606 respectively). Significance codes: *** 0.001; ** 0.01; * 0.05; none = not significant. Error bars = SE.

level. Individuals in these early life stages are too small for cellular and physiological-level experimentation, but do experience increased mortality, a population level effect, as a result of heavy metal contamination (for example, Fathallah et al., 2013; Boukadida et al., 2016). Nonetheless, the results imply that a focus on physiological or cellular level effects could underestimate the effect of heavy metal pollution on a population or ecosystem, because of the same assumptions I made in my hypothesis.

4.5. Numerical moderators

Both concentration and duration as moderators were less significant than expected. Given that these moderators were not significant for Cu, one of the largest metal subsets, it is unlikely that the lack of significance of these moderators is due to insufficient sample size at finer resolutions within the nested study model. Still, as O'Brien and Keough (2014) point out, the lack of a strong dose-concentration correlation may be due to excessive between-study variance. The results underline the importance for individual biomonitoring studies of determining how effects change with concentration and time, and of not assuming a linear or even positive relationship. The results also raise several questions. First, would more significant results be obtained for concentration if studies measured how much of a heavy metal was bioaccumulated by the organism, rather than how the amount of metal added to the tank? Second, do concentration and duration interact as moderators? The amount of metal in an organism as opposed to the amount in the environment may provide a closer correlation with the effects of heavy metal contamination. Nonetheless, given that a number of factors can regulate the rate at which metals are bioaccumulated, and that the toxic effects do not increase linearly over time, the answers to these questions are likely not straightforward.

4.6. Comparison with other studies

The most recent comprehensive English-language review of heavy metal biomonitoring using marine bivalves is that of Zukyov et al. (2013). Although these authors focused mostly on biomonitoring techniques rather than magnitudes of effect, several of the conclusions they reached qualitatively are ones that this meta-analysis analyzed. The authors state that drastic effects of accumulated metals on bivalve health have not been documented even in areas of heavy pollution. Although other terminology in the review is defined and discussed very thoroughly, drastic effects are defined only through the examples of shell deformations and imposex (growth of male genitalia on females). The conclusion of no drastic effects is thus difficult to compare to the results of my analysis, which, although quantitatively derived, are dimensionless. That being said, individual studies compiled in this meta-analysis (especially Liu et al., 2014; Gamain et al., 2016) indicate that, in fact, qualitatively severe effects can occur. Nonetheless, Zukyov et al. (2013) are correct that adult bivalves show a remarkable ability to persist even in highly polluted environments (Kavun and Podgurskaya, 2009), hence their value as biomonitors!

This study can also be compared to the meta-analysis of heavy metal effects by O'Brien and Keough (2014), who examined the effects of diverse kinds of organic and inorganic pollution across many taxa in both the laboratory and the field. Due to sampling limitations, their formal meta-analysis focused on only Cu pollution, which had the largest number of papers, and they compared results across three levels of biological organization (individual, population, and community). The results of this study are in agreement with O'Brien and Keough (2014), in that both studies found a negative response to Cu. Both studies also found a negative dose-response relationship for copper, although different methods were used (this study included concentration as a numerical moderator, whereas O'Brien and Keough analyzed effect size vs. copper dose graphically).

This study and that of O'Brien and Keough (2014) used the same dimensionless measurement for effect (log of effect size) so that magnitudes of effect can be compared directly between the studies. In O'Brien and Keough's (2014) individual category, they estimated an ecological response magnitude of approximately −0.35, and in their population category they measured an ecological response magnitude of approximately −0.65. Their overall response magnitude was approximately −0.31 (values derived from graphs). In this study, physiological and population categories (corresponding respectively to individual and population categories in O'Brien and Keough's study: see Tables 5A & 5B for comparison) for Cu had ecological response magnitudes of 0.1301 and 0.4797 respectively, and the overall response magnitude for Cu was 0.3259. The difference in sign between our results is because my analysis used absolute values so there are no negatives (see methods for further explanation). These results are very similar, and the values obtained by O'Brien and Keough would still position Cu in the second rank of effects sizes among the six heavy metals evaluated here. Given that this study focused exclusively on marine bivalves, whereas O'Brien and Keough included studies of many marine invertebrates, and given that this study focused exclusively on mesocosm data while O'Brien and Keough included field studies, the

Table 5A
Biological responses categorized as individual, population, and community level in the meta-analysis of O'Brien and Keough (2014).

Biological responses categorized as individual, population or community responses.

Response category	Responses measured
Individual	Pathological, behavioural, bioaccumulation/ bioconcentration, survival, histological, molecular (including biomarkers, genes), larval settlement, growth, fertilization, fecundity or reproductive output
Population	Abundance (one taxon), reproductive output, fecundity, population growth
Community	Abundance (one or more taxon)[a], species richness, diversity, evenness

[a] Measured in the context of other individuals in the community.

Table 5B
Biological responses categorized as cellular, physiological, and population in my meta-analysis.

Level of biological organization	Response measured
Cellular	Total glutathione content, total hemocyte count, sodium concentration, potassium concentration, glucose concentration, phagocytic activity, phagocytic index, superoxide dismutase, % of hemocytes showing cytochrome oxidase activity, esterase activity, total antioxidant capacity, total oxidant status, ATPase activity, glutathione transferase activity, GSSG reductase activity, catalase activity, RNA/DNA ratio, lipid content, protein content, glycogen content, glutathione peroxidase, total glutathione, ratio reduced to oxidized glutathione, thiobarbaturic acid reactive substances, lysosomal stability, micronuclei frequency, lysosomal lipofuscin, lysosomal destabilization, lipid peroxidation, lysosomal latency, neutral lipid content, lysosome/cytoplasm volume ratio, glutathione transferase, total M O2, mitochondrial M O2, proton leak, hemocytes, zymosan uptake, lysozyme, RNA content, protonmotive force, nitrite accumulation, ATP levels, DNA breakage, gamma-glutamyl-cstein synthetase in gill, total oxiradical scavenging capacity
Physiological	Clearance rate, filtration rate, oxygen consumption rate, absorption, assimilation efficiency, % injured tubules/filaments, % necrotic tubules/filaments, energy reserves, sperm swimming speed, NH4-N excretion, O;N ratio, scope for growth, growth efficiency, digestive tubule dilation, digestive tubule breakdown, cilia loss in digestive diverticula, shell length, dry shell weight, dry byssus weight, fecal production rate, assimilation, dry tissue weight, average valve closing time
Population	Gonado-somatic index, sex ratio, larval abnormalities, larval developmental arrest, fertilization %, hatched larvae %, larval mortality

similarity between our overall results suggests that these results are robust.

5. Conclusions

Understanding the effects of heavy metal pollution on marine bivalves has important management implications. Knowledge of general trends is useful in prioritizing concerns and streamlining guidelines, thus ensuring that limited resources are employed as efficiently as possible. This meta-analysis generally confirmed overarching trends of metal toxicity, but demonstrates that toxic effects do not always follow intuitive patterns. The ranked effect size order of Pb > Hg > Cu > Zn > Cd > Ag was expected apart from the higher toxicity of Zn than Cd, but the toxicity ranking by level of biological organization, cellular < physiological < population, was the opposite of what was hypothesized. Whether or not a metal is an essential micronutrient was also found to be a poor predictor of toxicity. In the future, overlapping the data from this meta-analysis with information on marine bivalves' geographic ranges and global patterns of marine heavy metal pollution could be extremely helpful in determining which areas, species, or ecosystems are under the most strain and directing management efforts accordingly, whether at a local, national, or global level.

Having read over hundreds of studies measuring the effect of heavy metals on marine bivalves, I can confidently make several recommendations for future biomonitoring studies, and reinforce recommendations made by other authors. Bivalves as biomonitors are an integrated tool and should be used as one of multiple lines of evidence, as emphasized by O'Brien and Keough (2014) and Zukyov et al. (2013). The numerous biotic and abiotic factors that contribute to the rate at which heavy metals accumulate, and to the detrimental effects of these metals, indicate that results obtained from biomonitoring must be examined in conjunction with other measurements in order to prevent error from confounding effects. Mesocosm conditions should always be explicitly described (i.e. water temperature, salinity). Also, many laboratory studies measured either bioaccumulation (ex. concentration in various organs) or biological effect of various metal concentrations. Given that effect likely correlates more closely to the amount of metal taken up than to the amount added to a mesocosm, I would strongly recommend measuring both of these variables in future studies. Doing so may elucidate dose-response relationships that are often unclear, inconsistent, or ambivalent when compared with concentration of metal added. Lastly, given that the magnitude of effect size (Pb > Hg > Cu > Zn > Cd > Ag) is not the same as the frequency of the metal being studied (Cu > Cd > Zn > Ag > Pb > Hg), a qualitative analysis could underestimate the effect of heavy metal contamination on marine bivalves because the metals that are studied most frequently are not those that cause the most severe effects. Future studies should be careful not to confuse the two measures.

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Appendix A. List of studies used in meta-analysis

Title	First author	Date of publication	Journal
Comparison of intermittent and continuous exposures to cadmium in the blue mussel, *Mytilus edulis*: accumulation and sub-lethal physiological effects	Amachree	2013	Ecotoxicology and Environmental Safety
Metal accumulation, filtration and O_2 uptake rates in the mussel *Perna perna* (Mollusca: Bivalvia) exposed to Hg2q, Cu2q and Zn2q	Anandraj	2002	Comparative Biochemistry and Physiology Part C
Co-exposure to n-TiO_2 and Cd^{2+} results in interactive effects on biomarker responses but not in increased toxicity in the marine bivalve *M. galloprovincialis*	Balbi	2014	Science of the Total Environment
Filtration rate, assimilation and assimilation efficiency in *Crassostrea virginica* (Gmelin) fed with *Tetraselmis suecica* under cadmium exposure	Barrera-Escorcia	2010	Journal of Environmental Science and Health Part A
Inhibition of embryo development of the commercial bivalves *Ruditapes decussatus* and *Mytilus galloprovincialis* by trace metals; implications for the implementation of seawater quality criteria	Beiras	2004	Aquaculture
Early and efficient induction of antioxidant defense system in *Mytilus galloprovincialis* embryos exposed to metals and heat stress	Boukadida	2017	Ecotoxicology and Environmental Safety

High sensitivity of embryo-larval stage of the Mediterranean mussel, *Mytilus galloprovincialis* to metal pollution in combination with temperature increase	Boukadida	2016	Marine Environmental Research
Heavy metals and glutathione metabolism in mussel tissues	Canesi	1999	Aquatic Toxicology
Effects of heavy metals on oxygen consumption and ammonia excretion in green-lipped mussels (*Perna viridis*)	Cheung	1995	Marine Pollution Bulletin
Cadmium-induced oxidative stress in the bivalve mollusk *Modiolus modiolus*	Dovzkenko	2005	Russian Journal of Marine Biology
Macromolecule oxidation and DNA repair in mussel (*Mytilus edulis L.*) gill following exposure to Cd and Cr(VI)	Emmanouil	2007	Aquatic Toxicology
Combined toxicity of lead and cadmium on embryogenesis and early larval stages of the European clam *Ruditapes decussatus*	Fathallah	2013	Environmental Engineering Science
Toxicity of Hg, Cu and Zn on early developmental stages of the European clam (*Ruditapes decussatus*) with potential application in marine water quality assessment	Fathallah	2010	Environmental Monitoring and Assessment
The relative sensitivity of sperm, eggs and embryos to copper in the blue mussel (*Mytilus trossulus*)	Fitzpatrick	2008	Comparative Biochemistry and Physiology
Esterase activity (EA), total oxidant status (TOS) and total antioxidant capacity (TAC) in gills of *Mytilus galloprovincialis* exposed to pollutants: analytical validation and effects evaluation by single and mixed heavy metal exposure	Franco	2016	Marine Pollution Bulletin
Combined effects of pollutants and salinity on embryo-larval development of the Pacific oyster, *Crassostrea gigas*	Gamain	2016	Marine Environmental Research
Influence of metal exposure on metallothionein synthesis and lipid peroxidation in two bivalve mollusks: the oyster (*Crassostrea gigas*) and the mussel (*Mytilus edulis*)	Géret	2002	Aquatic Living Resources
Effects of silver nanoparticles exposure in the mussel *Mytilus galloprovincialis*	Gomes	2014	Marine Environmental Research
Interactive effects of pH and metals on mitochondrial functions of intertidal bivalves *Crassostrea virginica* and *Mercenaria mercenaria*	Ivanina	2013	Aquatic Toxicology
Immunomodulation by the interactive effects of cadmium and hypercapnia in marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*	Ivanina	2014	Fish and Shellfish Immunology
Interactive effects of copper exposure and environmental hypercapnia on immune functions of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*	Ivanina	2016	Fish and Shellfish Immunology
Toxicity of metals to the bivalve *Tellina deltoidalis* and relationships between metal bioaccumulation and metal partitioning between seawater and marine sediments	King	2010	Environmental Contamination and Toxicology
Physiological and cellular responses to copper and mercury in the green mussel *Perna viridis* (Linnaeus)	Krishnakumar	1990	Aquatic Toxicology
Effect of chronic sublethal exposure of major heavy metals on filtration rate, sex ratio, and gonad development of a bivalve species	Liu	2014	Bulletin of Environmental Contamination and Toxicology
Embryotoxic and genotoxic effects of heavy metals and pesticides on early life stages of Pacific oyster (*Crassostrea gigas*)	Mai	2012	Marine Pollution Bulletin
Effects of copper and cadmium exposure on functional responses of hemocytes in the clam, *Tapes philippinarum*	Matozzo	2001	Environmental Contamination and Toxicology
Effect of copper on the scope for growth of clams (*Tapes philippinarum*) from a farming area in the Northern Adriatic Sea	Munari	2007	Marine Environmental Research
Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (*Mytilus trossolus*) and the protective effect of dissolved organic carbon	Nadella	2009	Comparative Biochemistry and Physiology
Effects of sublethal Zn^{++} and Cd^{++} concentrations on filtration rate, absorption efficiency and scope for growth in *Donax trunculus* (Bivalvia; Donacidae)	Neuberger-Cywiak	2007	Environmental Contamination and Toxicology
Interactions of silver, cadmium, and copper accumulation in green mussels (*Perna viridis*)	Ng	2007	Environmental Toxicology and Chemistry
Mussel microsomal Na + -Mg2 + -ATPase sensitivity to waterborne mercury, zinc and ammonia	Pagliarani	2009	Comparative Biochemistry and Physiology
Embryo-larval tolerance of *Mytilus galloprovincialis*, exposed to elevated seawater metal concentrations - II. Stage-specific fluctuations in sensitivity towards Zn and Cd and their bioaccumulation into veliger larvae	Pavicic	1994	Comparative Biochemistry and Physiology
Accumulation of cadmium and bioenergetics in the mussel *Mytilus edulis*	Poulsen	1982	Marine Biology
Modulations in antioxidant enzymes in different tissues of marine bivalve *Perna viridis* during heavy metal exposure	Prakash	1995	Molecular and Cellular Biochemistry
Dysfunctions of the translational machinery in digestive glands of mussels exposed to mercury ions	Pytharopoulou	2013	Aquatic Toxicology
Comparative sensitivity of gametes and early developmental stages of a sea urchin species (*Echinometra mathaei*) and a bivalve species (*Isognomon californicum*) during metal exposures	Ringwood	1992	Environmental Contamination and Toxicology
Tissue injury and cellular immune responses to cadmium chloride exposure in the common mussel *Mytilus edulis*: modulation by lipopolysaccharide	Sheir	2010	Environmental Contamination and Toxicology

Effect of pollution history on immunological responses and organ histology in the marine mussel *Mytilus edulis* exposed to cadmium	Sheir	2013	Environmental Contamination and Toxicology
Evaluation of the effects of the metals Cd, Cr, Pb and their mixture on the filtration and oxygen consumption rates in *Catarina* scallop, juveniles	Sobrino-Figueroa	2014	Journal of Environmental Biology
Alterations of valve closing behavior in juvenile Catarina scallops (*Argopecten ventricosus* Sowerby, 1842) exposed to toxic metals	Sobrino-Figueroa	2009	Ecotoxicology
Effects of chronic copper exposure on the green mussel *Perna Viridis*	Sze	2000	Marine Biology
Exposure–dose–response of *Anadara trapezia* to metal contaminated estuarine sediments. 1. Cadmium spiked sediments	Taylor	2012	Aquatic Toxicology
Exposure–dose–response of *Anadara trapezia* to metal contaminated estuarine sediments. 2. Lead spiked sediments	Taylor	2012	Aquatic Toxicology
In viva effects of copper on the calcium homeostasis mechanisms of mussel gill cell plasma membranes	Viarengo	2004	Comparative Biochemistry and Physiology
Sublethal effect of silver and chromium in the green mussel *Perna viridis* with reference to alterations in oxygen uptake, filtration rate and membrane bound ATPase system as biomarkers	Vijayavel	2007	Chemosphere
Sub-lethal effects of cadmium and copper on RNA/DNA ratio and energy reserves in the green-lipped mussel *Perna viridis*	Yeung	2016	Ecology and Environmental Safety
Aquatic Toxicology, Tissue specific responses of oysters	McCarthy	2013	*Crassostrea virginica*, to silver nanoparticles

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2018.04.068.

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