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D1.4 Meta analysis of the driver databases and development of new parameterisations relevant to the ecosystem models

Part D: Pollution

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1. Introduction

The principal aim of the pollution meta-analysis was to examine the existing literature for potential parameterizations and thereby enable a better understanding of the influence of key pollutants occurring in the marine environment on organism vital rates as well as where possible the combined effects of temperature and these pollutants on lower trophic level key players. As a review of the vast number of contaminants occurring in the marine environments is beyond the scope of this program we focus on contaminants chosen in the experimental component of MEECE.

2. General Overview

2.1 Temperature

As most organisms living in the marine environment are ectotherms temperature is one of the most influential agents on factors such as mortality (e.g. Blaxter 1960), reproduction (e.g. Holste and Peck 2006), development rate (e.g. Heinle et al. 1969, Miller et al. 1977, Klinkhardt and Biester 1984), growth (e.g. Høie et al. 1999), swimming speed and activity (e.g. Batty et al. 1993). For a species, populations existing at the optimal temperature populations are, in general considered to be more fit with both increased reproduction and development (Kingsolver and Huey 2008). For example, egg production of *Acartia tonsa* is highly dependent on temperature with egg production increasing to an optimal temperature at 24.7°C where the highest egg production were measured then decreasing until the species maximum temperature of 34°C where all adults died (Holste and Peck 2006).

Development time is in general considered to decrease with temperature (Kingsolver and Huey 2008). For example herring (*Clupea harengus*) embryos required 6 days to hatch at 14°C while at 4°C the development time was 26 days (Klinkhardt and Biester 1984). Similarly, the copepod *Acartia clausi* required 13 days to develop to the adult stage at 20°C while at 15°C after 17 days the copepods had advanced the last copepodite stage before adult (Miller et al. 1977). Species with a short life span (e.g. copepods with a shorter development time) the generation time will also be reduced (Kingsolver and Huey 2008).

Temperature also has an impact upon organism body size with a larger body size at lower temperatures. These larger individuals are, in general considered to show better fitness; however, organism body size decreases with increasing temperatures (Kingsolver and Huey 2008). For example the length of copepods (*Acartia tonsa*) and the herring larvae have been shown to decrease with increasing temperatures (Miller et al. 1977, Peck et al. in press) making them less competitive. The interaction between temperature and size led Kingsolver and Huey (2008) to state “*Bigger is better, And hotter makes you smaller, Hotter is better*” This conflicting statement is based on the fact that increased temperature can impact both on the organism size which may be smaller with lower fitness expected while metrics such as development and reproduction may increase thereby increasing the organisms potential for survival (Kingsolver and Huey 2008).

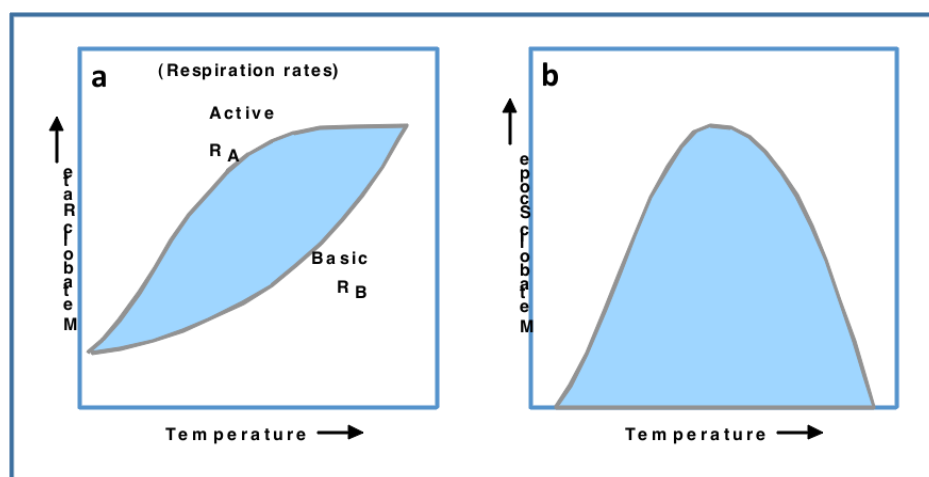


Figure 1. Conceptual diagram of metabolism; active metabolism and basic metabolism rate at increasing temperatures (a), the scope of metabolism at increasing temperatures (b), based on Fry (1957).

Temperature drives metabolism influencing almost all physiological processes in an organism. The term Q_{10} of an organism, roughly a doubling in vital rate (e.g. respiration) with an increase of 10°C reflects this relationship.

Metabolism can be divided into three categories; basal or resting metabolism, active metabolism when an organism is swimming or searching for food and feeding metabolism or digestion often termed specific dynamic action (SDA). Metabolism continuously increases with temperature with the metabolic scope of an organism increasing to a maximum then to decreasing with increasing temperatures (e.g. figure1) As these two different trends do not follow each other temperature, is often considered the determining factor for organism fitness and survival (Peck and van der Veer 2010).

As metabolism increases with increasing temperatures which then dictates that organisms require more energy for maintenance. Resultant changes in metabolism could also influence the impacts of different contaminants. Example with a higher metabolism another stressor such as exposure to a contaminant could potentially increase the stress. Furthermore metabolism could impact upon bio-activation or detoxification depending on the compound and the species (Noyes et al. 2009). Finally it has been shown than temperature influences the accumulation and elimination of contaminant (Yang and Chen 1996, Heugens et al. 2003, Mubiana and Blust 2007).

2.1.1 The combined effect of temperature and contaminants

The interaction between contaminants and temperature is complex with a wide range of responses to the large range of compounds which are available in the environment. Six possible responses can be proposed when organisms are exposed to contaminants at different temperatures shown in the conceptual model figure 2 (modified from Sokolova and Lannig 2008). Acute experiments performed on crustaceans using two to three different temperatures and exposure to copper,

mercury, chromium, zinc, nickel, lead or the insecticide Tebufenozide being the most common (e.g. McLusky and Hagerman 1987, Song et al. 1997, Bat et al. 2000, Tsui and Wang 2006). Those studies have shown an increase in toxicity when temperature increases.

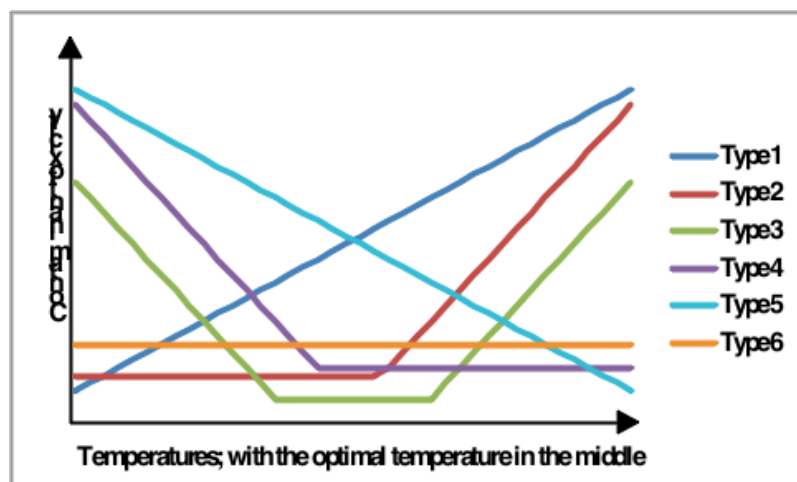


Figure 2. Conceptual model of temperatures influence on contaminants toxicity modified from Sokolova and Lannig (2008). Type 1; the toxicity increases continuously with the temperature. Type 2; the toxicity stays constant with increasing temperatures until about the optimal temperature where the toxicity increases with increasing temperatures. Type 3; the toxicity decrease with temperature until a certain point close to the optimal temperature where the toxicity stay constant for then to start to increase again. Type 4; the toxicity decreases with increasing temperatures for then at about the optimal temperature to stay constant. Type 5; the toxicity decreases continuously with increasing temperature. Type 6; the toxicity stay the same independent on the temperature.

In the next part detailed examples of experiments with the combined effect of contaminants and temperatures are involved. The criteria for choosing those experiments are that they include more than three temperatures.

Copper and nonylphenol as example contaminants

2.1.1.1 Copper

There are many different anthropogenic sources which introduce copper into the marine environment; these include industrial discharge, antifouling paint, mining activities and air pollution (Dippner and Pohl 2004, Andrade et al. 2006, Parks et al. 2010). For example, in 1990, 1279 tonnes of copper entered the Baltic Sea from riverine sources with 1205 tonnes from the air (Dippner and Pohl 2004). *In situ* measurements of copper have identified copper concentrations in the Baltic Sea of 0.1-0.6 $\mu\text{g l}^{-1}$ (Dippner and Pohl 2004, Strady et al. 2008) and in the North Sea at 0.1-0.9 $\mu\text{g l}^{-1}$ (Law et al. 1994). Copper is considered to be one of the contaminants having a significant influence on marine organisms (Sunda and Guillard 1976, Taylor et al. 1985). Inhibition of the growth rate of different microalgae, i.e. *Tetraselmis chuii*, *Rhodomonas salina*, *Chaetoceros sp.*, *Isochrysis galbana* and *Nannochloropsis gaditana*, exposed for 72 hours to a range of copper concentrations has been determined by Debelius et al (2009).

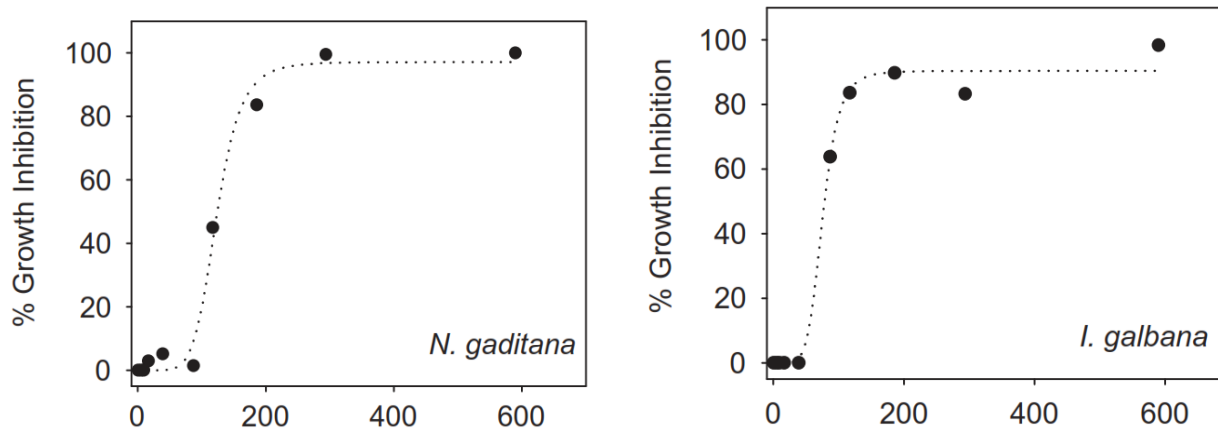
Exponentially growing populations of each microalgal species have been exposed to ten progressively increasing nominal concentrations of copper, including a control (0, 5, 10, 20, 40, 80, 120, 200, 300 and 600 $\mu\text{g L}^{-1}$). After 72h of copper exposure, all samples have been analysed by flow cytometry and EC_{50} values have been calculated (Table 1).

Table 1. Concentration of Cu able to induce 50% of inhibition in the growth rate of the different tested microalgae species (from Debelius et al 2009).

Microalgae species	EC_{50} ($\mu\text{g L}^{-1}$)
<i>Nannochloropsis gaditana</i>	137
<i>Isochrysis galbana</i>	58
<i>Chaetoceros sp.</i>	88
<i>Rhodomonas salina</i>	48
<i>Tetraselmis chuii</i>	330

In the order of increasing sensitivity to copper these 5 species were ranked by *T. chuii*, *N. gaditana*, *Chaetoceros sp.*, *I. galbana* and *R. salina*.

The different curves of algal growth rate in the range of tested copper concentrations have also been reported for each studied species (figure 3).



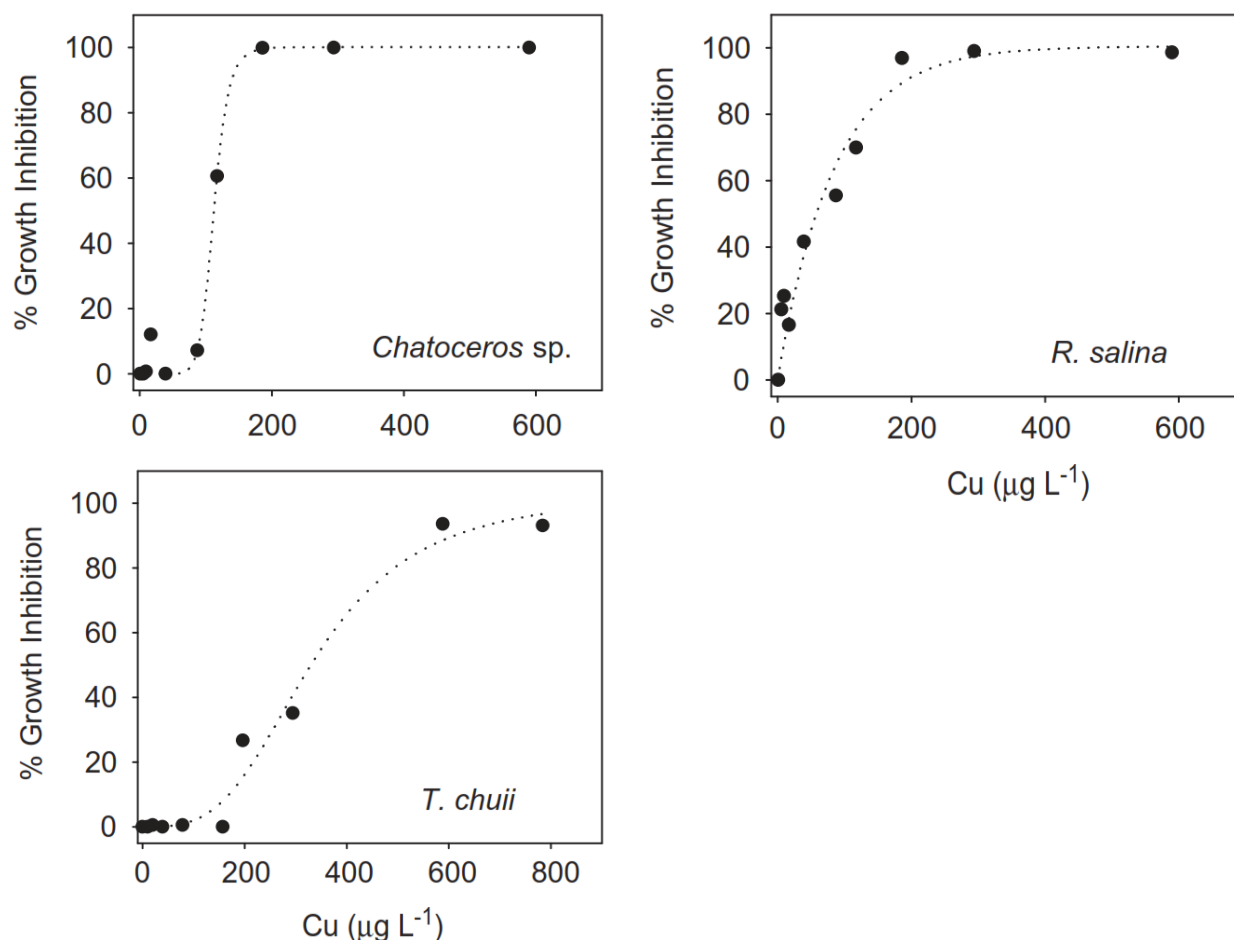


Figure 3. Curves describing growth inhibition in the range of tested copper concentration (from Debelius et al 2009).

Debelius et al (2009) reported copper-induced inhibition of algal growth in other marine and freshwater microalgae from the scientific literature (table 2).

Table 2. Copper EC₅₀ on growth rate of selected algal species.

Microalgae species	Habitat*	EC ₅₀ (µg L ⁻¹)	References
<i>Aphanizomenon gracile</i>	FW	64	Lüderitz & Nicklish 1989
<i>Chlorella sp.</i>	FW	8	Franklin et al 2001
<i>Chlorella sp.</i>	FW	735	Franklin et al 2002
<i>Dunaliella tertiolecta</i>	M	530	Levy et al 2008
<i>Dunaliella tertiolecta</i>	M	1000	Franklin et al 2001
<i>Phaeodactylum tricornutum</i>	M	8	Levy et al 2008
<i>Phaeodactylum tricornutum</i>	M	10	Franklin et al 2001
<i>Rhodomonas salina</i>	M	30	Moreno-Garrido et al 1999
<i>Pseudokirchneriella subcapitata</i>	FW	8	Franklin et al 2001

<i>Pseudokirchneriella subcapitata</i>	FW	75	Franklin et al 2002
<i>Tetraselmis sp.</i>	M	47	Levy et al 2008
* from Guiry 2011.			

Data show a wide range of sensitivity for the different algal species with EC_{50} ranging from $7.35 \mu\text{g L}^{-1}$, as reported for *Chlorella sp.* by Franklin et al (2002) to 1 mg L^{-1} , as reported for *Dunaliella tertiolecta* by Franklin et al (2001).

Selected data (tables 1 and 2) have been utilized to determine a Species Sensitivity Distribution (SSD) for microalgae exposed to copper. SSDs are cumulative probability distributions of toxicity values for multiple species (Posthuma et al 2002).

SSD for algal growth rate has been determined following the method proposed by US EPA (2005) (figure 4). When more than one value of EC_{50} is available for the same species (i.e. *Dunaliella tertiolecta*), the geometric mean is used in the estimation of SSD (US EPA 2005).

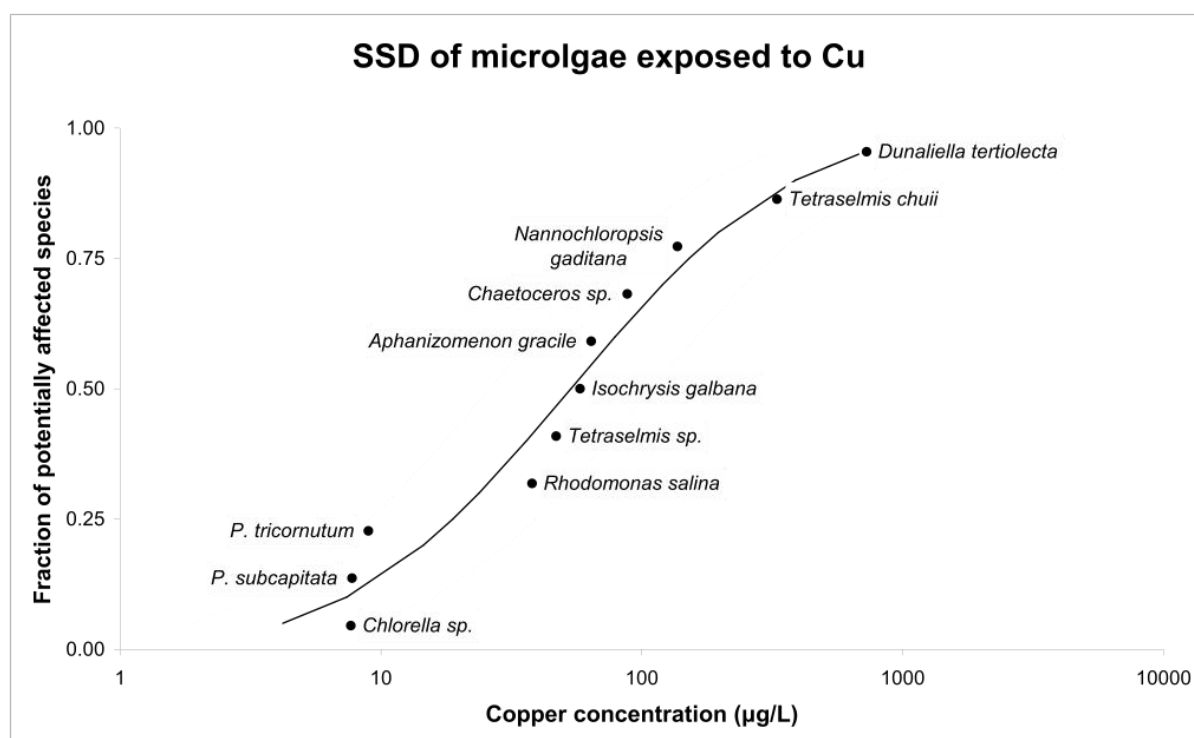


Figure 4. Species sensitivity distribution for algal growth rate in response to copper: in the curve (solid line) the percent of potentially affected species (y-axis) is related to the logarithm of copper concentration ($\mu\text{g L}^{-1}$) (x-axis); dotted lines determine the 95% confidence interval; dots report species used to fit the curve.

SSD curve has been fitted considering a linear relationship between the Probit function of potentially affected species (i.e. the inverse cumulative distribution function associated with a

normal distribution with $\mu=5$ and $\sigma=1$) and the logarithm of the concentration of copper (equation 1).

$$\text{Probit (AS\%)} = \alpha \times \log_{10}[\text{Cu}] + \beta \quad \text{Equation 1}$$

Where

AS% is the percent of potentially affected species;

α is the slope;

β is the intercept.

Fitting the data concerning copper effects on algal growth rate it is possible to estimate slope and intercept ($R^2 = 0.95$):

$$\alpha = 1.49$$

$$\beta = 2.43$$

For environmental risk assessment, the chemical concentration that may be used as a hazard level can be extrapolated from an SSD using a specified percentile of the distribution.

Applying the SSD curve it is possible to determine the copper concentration corresponding to a particular fraction of potentially affected algae (table 3).

Table 3. Copper concentrations predicted to affect selected fractions of algae species as calculated applying SSD for algal growth rate (equation 1).

Fraction of potentially affected species	Predicted copper concentration ($\mu\text{g L}^{-1}$)
5 %	4,21
10 %	7,37
25 %	18,85
50 %	53,46
75 %	151,63
90 %	387,53
95 %	679,50

The derivation of SSD is recognised as a robust tool to estimate the link from ecotoxicological data and potentially affected species. However, the goodness of the prediction is strongly dependent on data used to extrapolate the curve. In particular, the number of tested species as well as the determined ecotoxicological parameters utilised in the definition of SSD is fundamental to get a good prediction.

Finally, it must be stressed that studying the adverse effects of pollution in the field, the number of toxic chemicals in a contaminated site that can act against biota is always higher than one, rendering the application of the SSD-approach a simplification of reality, because derived from effects on a single compound, and not considering the complex interactions in a pollutants mixture (i.e. synergistic, antagonistic, additive, etc).

Examination of the role of copper on marine fish species has also identified that sensitivity is species specific. Different fish species under the same experimental conditions have shown different sensitivity to copper hence a general rule cannot be applied (McKim et al. 1978, Besser et al. 2005).

Several studies have been performed examining the effect of copper on herring embryos. For example Blaxter (1977) in a study on Atlantic herring observed a decrease in % fertilised eggs when exposed to copper at $900\mu\text{g l}^{-1}$. In studies examining the effect of copper on hatch success almost no hatch success occurred at $90\mu\text{g l}^{-1}$ for the Atlantic herring (Blaxter 1977) while for Pacific herring 100% mortality occurred at $45\mu\text{g l}^{-1}$ (Rice and Harrison 1978). These differences could have been due to different experimental protocols as in the Pacific herring experiment copper concentrations were maintained at a stable level due to the use of a flow through system while in the case of the Atlantic herring experiment a semi static setup was used where the water was changed at intervals at 4-6 days. Hence, the experimental protocols used in these two experiments may be the explanation in the different sensitivities of the two populations (Blaxter 1977, Rice and Harrison 1978). The effect of copper exposure on the length of newly hatched Atlantic herring has also been examined and was observed to decrease at a copper concentration of $30\mu\text{g l}^{-1}$ (Abbasi and Sheckley 1995). The impact of copper on larval abnormalities is more complex, 70% Atlantic herring had of severe abnormalities at $30\mu\text{g l}^{-1}$ when the eggs were copper exposed immediately after fertilisation, while when the eggs were exposed four days after fertilisation the larvae were not malformed (Blaxter 1977). These results suggest that copper is more harmful early in embryo development indicative of a higher sensitivity during early development and/or when an egg is hydrating after fertilisation. During this period the egg chorion is more permeable which can lead to a higher copper uptake during this stage (Jeziarska et al. 2009). Studies on the effect of copper on respiration of herring eggs have not been performed however, experiments on seven month old common carp and one month old rainbow trout exposed to $100\mu\text{g l}^{-1}$ copper have shown a decrease in oxygen consumption compared to the control (Jeziarska and Sarnowski 2002).

2.1.1.2 *Nonylphenol*

In the North Sea, off shore oil activity introduces contaminants such as alkylphenols into the environment. Due to the large volumes of produced water discharged from the platforms large

amounts of alkylphenols are released, roughly 323 tonnes of alkylphenols in 2009 (OLF 2010). Measurements on the produced water from different platforms have identified high concentrations of a wide range of alkylphenols with a total concentration from the different platforms between 0.25-35.0 mg l⁻¹, where nonylphenol was measured up to 3.8 ng l⁻¹ (Boitsov et al. 2007). Measurements of alkylphenols in the seawater around a platform in the North Sea showed concentrations of alkylphenols from 20-55 ng l⁻¹. In a reference site 100 km from the platform had a concentration of alkylphenols at 11 ng l⁻¹ (Harman et al. 2009). Furthermore nonylphenol is a chemical which is used widely in industry having a production in 1997 in the EU of 73500 tonnes (HELCOM 2002). The major source of nonylphenol has been the production of nonylphenol ethoxylates which has been used broadly e.g. as emulsifiers and polymers/plastics (HELCOM 2002, Soares et al. 2010). Most of the nonylphenol entering the marine environment comes from the degradation of nonylphenol ethoxylates from industrial wastewater (HELCOM 2002). A concentration of 330 µg l⁻¹ nonylphenol has been measured in wastewater discharged into the river Aire, England. Downstream from the source, a concentration of 180 µg l⁻¹ was observed (Blackburn and Waddock 1995). The European Water Framework Directive (WFD) has classified nonylphenol as a priority toxic compound. As a result, concentrations of nonylphenol have been monitored along with 35 other compounds in 122 rivers in the EU. The average concentration of nonylphenol in these rivers was found to be 134 ng l⁻¹ (Loos et al. 2009). In coastal seas concentrations of nonylphenol has been measured for example; in Wismar Bay, Baltic Sea concentrations of 1.3-21.2 ng l⁻¹, in the German Bight, of the North Sea concentrations between 0.09-1.4 ng l⁻¹ while one km outside Barcelona, Spain concentrations of 300-500 ng l⁻¹ has been observed (Petrovic et al. 2002, Beck et al. 2005, Xie et al. 2006).

Measurements of nonylphenol in different levels in the food chain have shown that no bioaccumulation occurs despite nonylphenol having a high log K_{ow} (Ahel et al. 1993, Hu et al. 2005). This can be explained by a high elimination process. T_½ has been found to be less than a day for the common shrimp (*Crangon Crangon*) and the three-spined stickleback (*Gasterosteus aculeatus*) while for the blue mussel (*Mytilus edulis*) T_½ was about two days (Ekelund et al. 1990). Previous experiments have been performed with different species of nonylphenol on a range of different organisms. For example, one study examined the effect of p-nonylphenol in a flow through system on nine different species of marine organisms from three phyla; bivalves (*Mulinia lateralis* embryo), crustaceans (*Dyspanopeus sayi* zoea 4th and 5th, *Leptocheirus plumulosus* adult, *Americamysis bahia* postlarvae 1-day, *Palaemonetes vulgaris* larvae 2-day, *Homarus americanus* zoea first stage) and fish (*Cyprinodon variegatus* juvenile, *Menidia beryllina* juvenile, *Pleuronectes americanus* larvae 2-day). LC₅₀ after 96 hours showed a wide range of effect concentrations with the Flounder *P. americanus* (two days old larvae) found to be the most

sensitive with a LC_{50} value at $17\mu\text{g l}^{-1}$ and the mud crab *D. sayi* (zoea stage 4-5) to be the most tolerant with a LC_{50} at $>195\mu\text{g l}^{-1}$ (figure 5) (Lussier et al. 2000).

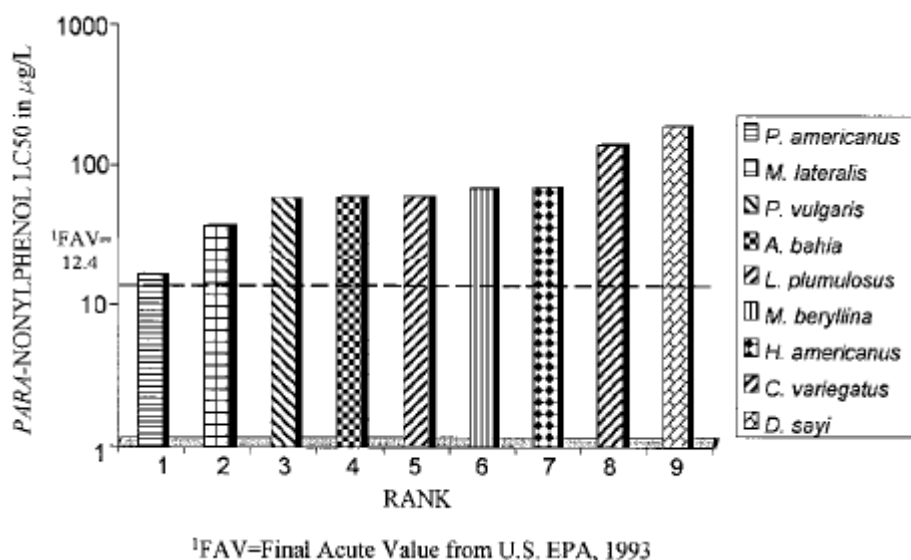


Figure 5. LC_{50} values after 96 hours of exposure from three different phyla (bivalve, crustaceans and fish) (from Lussier et al. 2000).

Survival of copepods exposed to nonylphenol has also been examined with inconclusive results. The marine copepod *Tisbe battagliai* had a 6% survival to maturity when exposed to $62\mu\text{g l}^{-1}$ nonylphenol however no effect at $31\mu\text{g l}^{-1}$ (Bechmann 1999). While for the copepod *Eurytemora affinis* nauplii at 96h LC_{50} at $38\mu\text{g l}^{-1}$ was observed (Forget-Leray et al. 2005).

In another study examining the effect of nonylphenol on development, the copepod *Eurytemora affinis* had the lowest effect concentration (LOEC) at $15\mu\text{g l}^{-1}$, while the mysid *Americamysis bahia* had a delay in development at a concentration of $30\mu\text{g l}^{-1}$ with a significant reduction in maturation at 14 days relative to the control (Forget-Leray et al. 2005, Hirano et al. 2009).

In freshwater organisms there is a wide range in sensitivity to nonylphenol. Employing a 96h LC_{50} the most sensitive organism tested was the amphipod *H. aztece* (juvenile 2mm) with a LC_{50} at $21\mu\text{g l}^{-1}$ whereas the adult snail *P. virgata* had a LC_{50} at $774\mu\text{g l}^{-1}$ (Spehar et al. 2010). A number of studies have been performed on *Daphnia* species where the reproduction of *Daphnia galeata* was observed to decrease by 50% at $65.2\mu\text{g l}^{-1}$ for the first generation exposed while the second generation had a 50% reduction at $81.5\mu\text{g l}^{-1}$ (Takanaka and Nakanishi 2002). Studies on *Daphnia* species produce different conclusions as well. In different studies on the reproduction of *Daphnia magna* the number of offspring decreased from 205 to 169 offspring parent⁻¹ when exposed to

56µg l⁻¹ (Comber et al. 1993) while in another study the number of offspring first decreased at 215µg l⁻¹ (Spehar et al. 2010). However, the results in these two studies could be due to the use of different isomers of nonylphenol.

2.1.1.3 Herbicides

During the past two decades the use of herbicides has been the primary method of weed control. Their agricultural use determined the introduction of these compounds into the (fresh water and marine) aquatic environment (reported environmental concentrations range from 0.003 to 1.65 µg/l .See Coors et al., 2006 and references therein), mostly through runoff. Herbicides can be detected in coastal marine environment, particularly in estuarine region, where watershed pollution is conveyed to the sea. Carafa et al. (2007) monitored (with a seasonal sampling frequency) the concentration of several herbicides (in water, sediments, macroalgae and clams) in the coastal Adriatic Sea around the Po river delta. Some “seasonal” variability was evident with the herbicides concentration peaking in the spring season, due to the coincident application period on the agricultural cultures and the increased rainfall characterising spring in northern Italy. Similar temporal pattern has been detected also in rivers (Pesce et al. 2008). However, the herbicide use in the aquatic environment can be deliberate, since herbicides of the triazine group have a toxicity effect on macroalgae and therefore are used as algicide on cooling systems. The potential consequences on non-target plants might be severe.

The adverse effect of herbicides on aquatic macroalgae has been documented by mesocosms (Coors et al. 2006), *in situ* (Eulaffroy and Vernet, 2003) and Laboratory (Jianyi M. et al., 2006) studies.

Herbicides of the triazine group act via inhibition of photosynthesis at the photosystem I (PSI) and photosystem II (PSII) levels by replacing PSI's ultimate electron acceptor or by blocking PSII-catalysed photosynthetic electron transport, therefore decreasing the electron flow rate from H₂O to NADP through PSII electron carriers (Powles et al. 1997; Eulaffroy and Vernet, 2003; Coors et al., 2006).

A general assessment of the herbicides effect on aquatic microorganisms has been provided by the extensive review of De Lorenzo et al. (2001). In general herbicides are known to have a toxic effect on microorganisms their effect acting on various biochemical processes (from photosynthesis to biosynthesis inhibition) In particular, herbicides of the triazine group have, similarly to aquatic macroalgae a negative effect on phytoplankton by acting on the photosynthetic system. In addition to the information obtained by the above quoted review, the more recent works of Macedo et al. (2008) on *Skeletonema Costatum* and of Magnusson et al. (2008) on benthic microalgae, confirmed such mechanism of action.

2.2 Contaminants and Primary producers

2.2.1 Microalgae

However, in a climate change perspective, possible alterations in pollutant-induced biological responses due to the action of environmental parameters should be considered.

In fact, environmental variables, such as temperature, can act on both chemical (Wolfe et al 1998) and biological parameters (Ahlgren 1987).

Temperature-induced alterations in algal growth are generally described applying Eppley equation (1972) or recent upgrades of the Eppley approach (Bissinger et al 2008, Moisan et al 2002).

Eppley equation suggests an exponential relationship between maximum algal growth and temperature (equation 2) (Eppley 1972).

Equation 2.

$$i_{MAX} = 0.59e^{0.0633T}$$

where

μ_{MAX} is the maximum attainable daily growth rate (d^{-1})

T is the temperature ($^{\circ}C$)

The influence of temperature on algal growth rate seems to have a species-specific relevance, as reported by Cairns et al (1978) (table 4).

Table 4. Mean specific growth rates obtained for each algal species at each temperature (from Cairns et al 1978).

Species	5°C	15°C	25°C	35°C
<i>Cyclotella meneghiniana</i>	Death	0.190	0.076	0.068
<i>Scenedesmus quadricauda</i>	0.264	0.224	0.143	negligible
<i>Chlamydomonas sp.</i>	Death	0.064	0.065	negligible
<i>Lyngbya sp.</i>	0.291	0.278	0.207	negligible

In particular, data reported by Cairns et al (1978) on the inhibition of algal growth on different algal species exposed to copper and temperature are reported in figure 6.

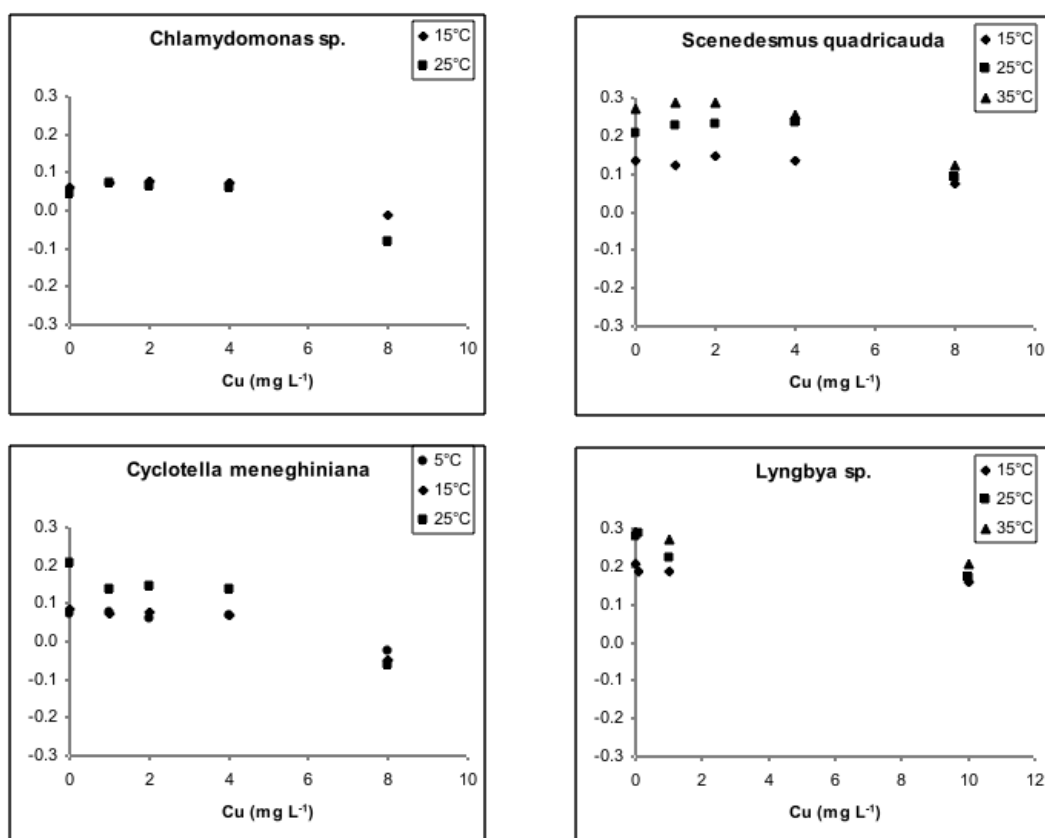


Figure 6. Effect of temperature on copper toxicity to selected algae (from Cairns et al 1978).

Cairns et al (1978) pointed out that higher copper concentrations inhibited growth at higher temperatures for *Chlamydomonas sp.*, *Scenedesmus quadricauda*, and *Cyclotella meneghiniana*, while the data on *Lyngbya sp.* were inconclusive (figure 6).

Data showed no temperature effects on copper sensitivity of *C. meneghiniana* between 5°C and 15°C, and a plateau existed in a range of Cu concentration between 1 and 4 mg L⁻¹. Copper-induced growth inhibition of *S. quadricauda* was clearer between 15°C and 25°C than between 25°C and 35°C.

In general, data from Cairns et al (1978) indicate that copper toxicity generally increases between 5°C and 15°C and between 15°C and 25°C for all the tested species; on the contrary, no increasing toxicity between 25°C and 35°C has been detected for *S. quadricauda*, while copper toxicity showed a decrease between 25°C and 35°C for *Lyngbya sp.*

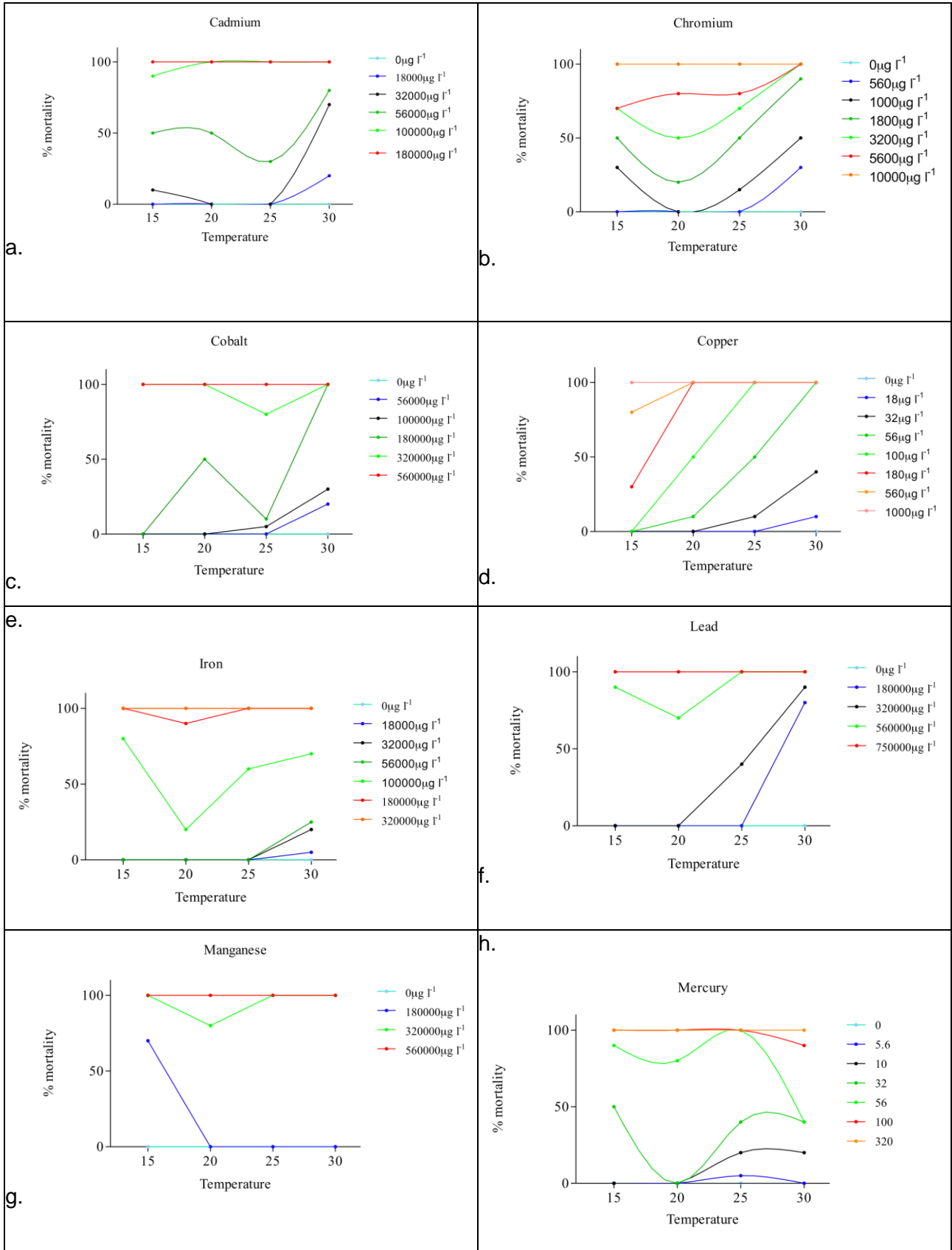
However, the interpretation of toxicity data should consider the differences in temperature tolerance of the tested algal species (table 4): in fact, some species are eurythermal while others are stenothermal.

In conclusion, although algal growth rate seems to be affected by temperature shifts, determining and modelling interferences of this environmental factor with sensitivity to contaminants on the basis of available bibliography is a challenging task, as the thermal effect has the potential to be function of autecological characteristic of each algal species composing natural populations.

2.3 Ecotox and higher trophic levels

Rathore and Khangarot (2002) exposed the freshwater sludge worm *Tubifex tubifex* to ten different metals (Cd, Cr, Co, Cu, Fe, Pb, Mg, Hg, Ni, Zn) at the temperatures 15, 20, 25 and 30°C. As there was no mortality of the controls at those temperatures all temperatures examined did not affect the mortality alone and it is impossible to know what would happened with metal toxicity if the temperatures were close to the limits of survival. For all metals the trends are shown with spline or point to point lines, as only four different results were available it was impossible to create equations for regression in this experiment (figure 7).

Figure 7 (continued). The % mortality of *Tubifex tubifex* exposed to different metals at different temperatures. Spline found for Hg and Ni and Point to Point lines for Mg and Zn. Modified from Rathore and Khangarot 2002. For each metal a different trend occurred. As no indication of variances was described in the paper all trends are with uncertainly. Copper and lead had a clear trend with an increased in toxicity with increasing temperatures. For zinc there could be the same trend with an increase in toxicity with increasing temperatures. For cobalt the increase in toxicity was clear at 30°C compared to the other temperatures. Cadmium and Chromium had a clear increase in toxicity at 30°C compared to the other temperatures. At 15°C there could be a trend to an increase in toxicity compared to 20 and 25°C. For the other five metals there was not a clear trend. For iron at the low concentrations (up to 56000µg l⁻¹) there was a trend to higher toxicity at 30°C compared to the other temperatures. At 100000 and 180000µg l⁻¹ there was a trend to less toxicity at 20°C compared to the other temperatures. Why this change in trends is difficult to explain and could be a part of the variability of the experiment. Nickel is difficult to read although the trend could be that there was no difference at the different temperatures. The curve for mercury is very complex maybe there was a trend with less toxicity at 20°C compared to the other temperatures.



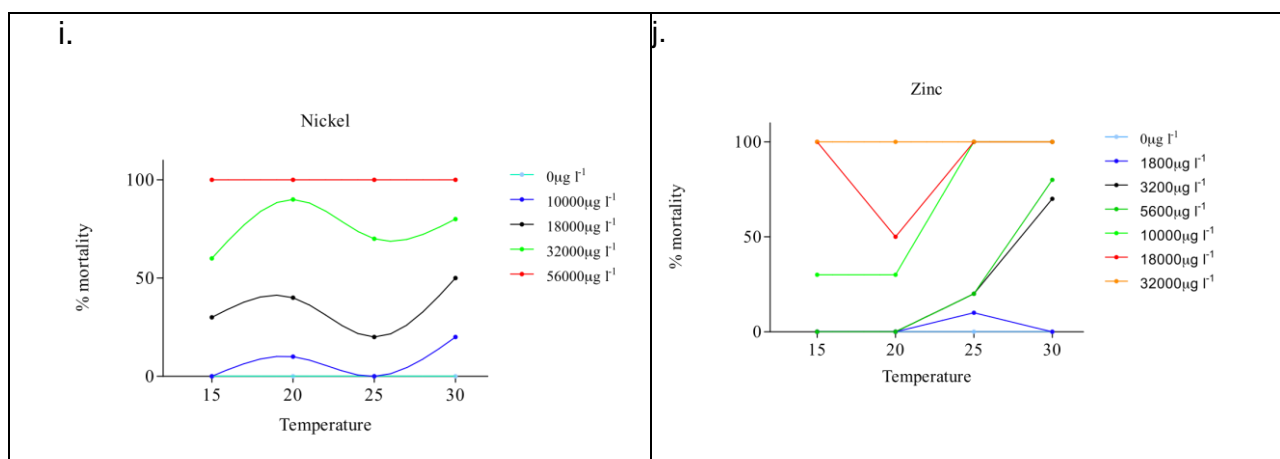


Figure 7. The % mortality of *Tubifex tubifex* exposed to different metals at different temperatures. Spline found for Cd and Cr and Point to Point lines for Co, Cu, Fe and Pb. Modified from Rathore and Khangarot 2002.

A study on the zebrafish, *Danio rerio* exposed to the pesticide diazinon at 26, 28, 30 and 33.5°C with the effects on a number of vital rate metrics including embryo heartbeat, embryo mortality, hatch success and malformation were assessed. The heartbeat increased with increased temperatures and increased toxicant until a certain concentration where the heartbeat started to decrease. This trend was the same independent on the temperature. Malformations were independent on temperature. For embryo mortality and hatch success the toxicity of diazinon increased with increased temperatures (figure 8). As there is only four points on the graph it was not possible to create regressions. This experiment showed how different metrics examined for one species had different trends. However, the overall outcome of the experiment showed a trend for increasing toxicity of diazinon with higher temperatures (figure 8) (Osterauer and Köhler 2008).

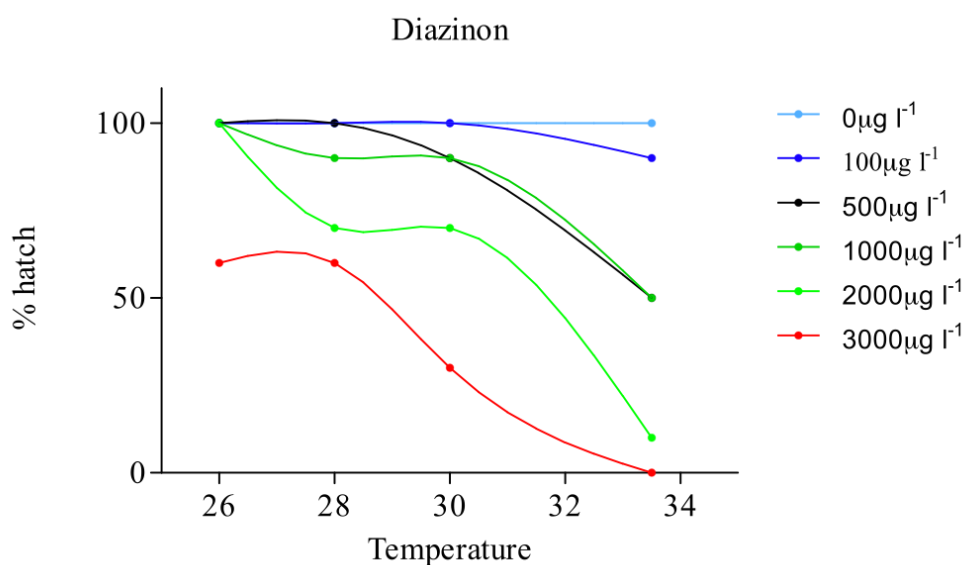


Figure 8. % hatch success for the zebrafish *Danio rerio* exposed to the pesticide diazinon at 26, 28, 30 and 33.5°C. The lines are spline from GraphPad Prism 5.

Persoone et al. (1989) exposed the waterflea *Daphnia magna*, rotifer *Brachionus plicatilis* and the brine shrimp *Artemia salina* to the potassium dichromate ($K_2Cr_2O_7$) which is common used in laboratory and sodium laurylsulphate which is common used in personal care products. The experiments were performed at different temperatures where the temperatures represent the range where the control organisms are not affected. At each temperature examined 24 hours LC_{50} were calculated. Furthermore for *D. magna* different water hardness were examined at the same time and for *B. plicatilis* and *A. salina* different salinities were examined at the same time. As there are only four points for *D. magna* and *B. plicatilis* the curves are spline. For *A. salina* a Boltzmann sigmoidal regression was used when exposed to potassium dichromate and a semilog line when exposed to sodium laurylsulphate (Figure 9). Best fit values and 95% confidence intervals are shown in table 5 and 6.

Boltzmann Sigmoidal: $Y, T = P_{max} + ((P_{min} - P_{max}) / (1 + e^{-(T - T_{50}) / Slope}))$

Boltzmann sigmoidal: $P(T) = P_{min} + ((P_{max} - P_{min}) / (1 + \exp(T_{50} - T / Slope)))$

T is the temperatures. (P_{min}) and (P_{max}) represent the minimum and maximum of the LC_{50} values. T_{50} defines the temperature where the middle range of LC_{50} occurred and slope represents the slope of the curve.

Semilog line: $Y(T) = 10^{-}$

Semilog line: $Y(T) = 10^{(slope * T + yintersept)}$

T is the temperatures. Slope defines the slope of the curve and y intercept is where the curve hit the y-axis.

Although the organisms were exposed at different conditions (water hardness or salinity) and the shapes of the curves were different all experiments showed the same trend with an increased toxicity of the contaminants with increased temperatures (figure 9). As no temperatures were stressing the organisms alone it is not known what would happened if the temperatures were close to the limits of the organisms.

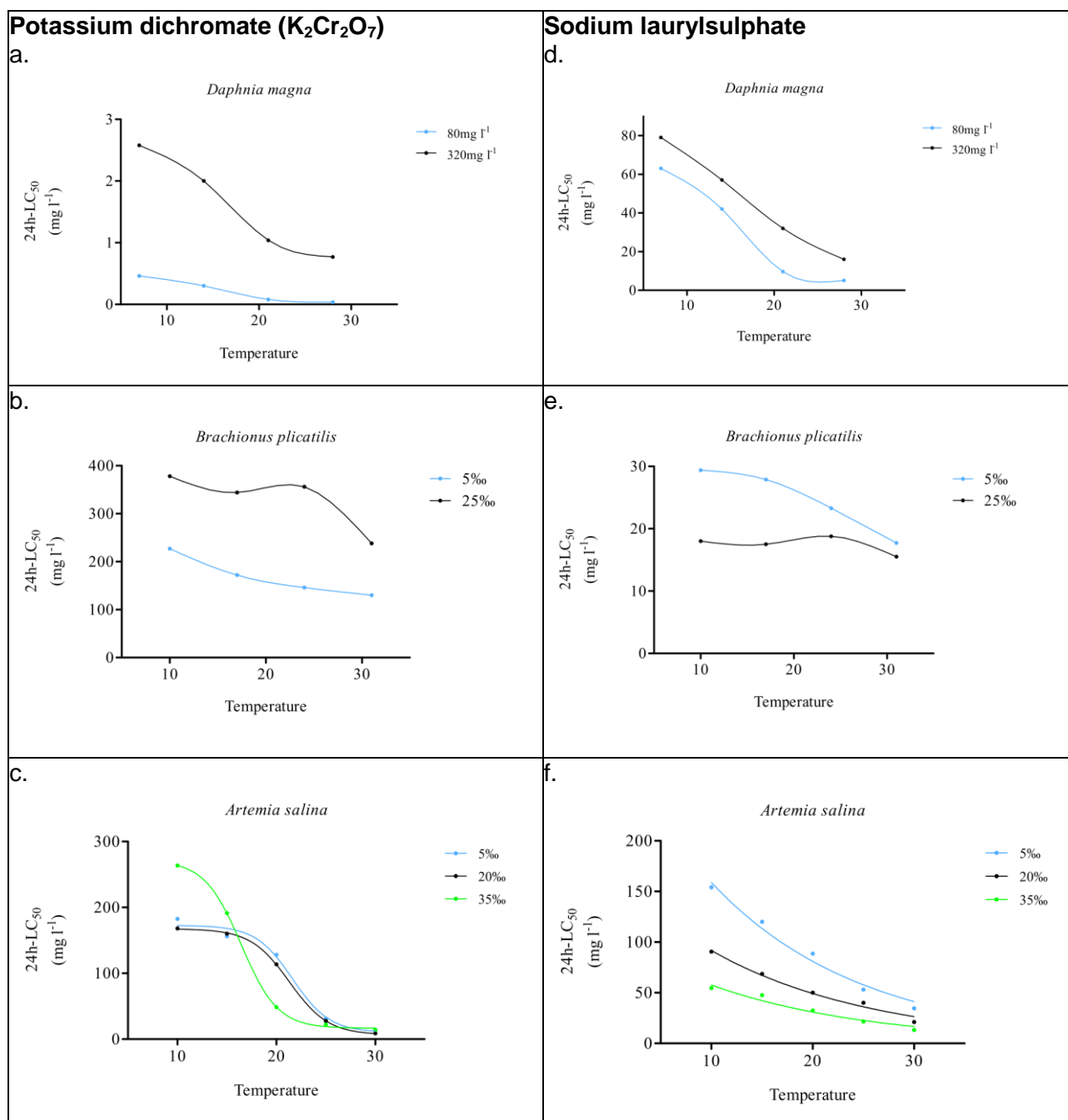


Figure 9. The 24 hours LC₅₀ for *Daphnia magna*, *Brachionus plicatilis* and *Artemia salina* when exposed to potassium dichromate a, b, c and sodium laurylsulphate d, e, f at different water hardness (*Daphnia magna*) and different salinities (*Brachionus plicatilis* and *Artemia salina*). For *Daphnia magna* and *Brachionus plicatilis* spline lines are shown. For *Artemia salina* exposed to potassium dichromate Boltzman sigmoidal regression lines are shown and when exposed to sodium laurylsulphate semilog lines are shown. Further information's about the best fit values and 95% confidence intervals are shown in table 5 and 6. Modified from Persoone et al. 1989.

Heugens et al. (2003) exposed *Daphnia magna* (less than 24 hours old neonates) to cadmium at a range of temperatures at 10-32°C. At the temperatures 10-26°C the control survival was at 100%, at 29°C the survival was about 90%, about 20% survival at 32°C and at 35°C there was a 100% mortality of the controls. For each temperature examined the LC₅₀ was calculated after 48 hours. In this detailed experiment there was a clear increase in toxicity of cadmium with increasing

temperatures until 23°C where the LC₅₀ was at a constant low level (figure 10). This could be explained with the increase in uptake at increasing temperatures. Which also were measured at the temperatures 10, 20 and 26°C. As there was a 100% survival of the controls at 10°C it is impossible to say what kind of trend there would happen if the temperatures decreased to close the temperature minimum. The regression fitted to the curve is a Boltzmann Sigmodial found in SigmaPlot Prism 5. The best fitted values and 95% confidence intervals are in table 5. Those parameters have to be read with carefulness as all points in the graph are based on approximations from a graph from the original paper (Heugens et al. 2003).

Boltzmann Sigmodial: $Y, T = P_{max} + ((P_{min} - P_{max}) / (1 + e^{-(T_{50} - T -$

Boltzmann sigmodial: $P(T) = P_{min} + ((P_{max} - P_{min}) / (1 + \exp(T_{50} - T / \text{Slope}))$

T is the temperatures. (Pmin) and (Pmax) represent the minimum and maximum of the LC₅₀ values. T50 is the temperature where the middle range of LC₅₀ occurred and slope represent the slope of the curve.

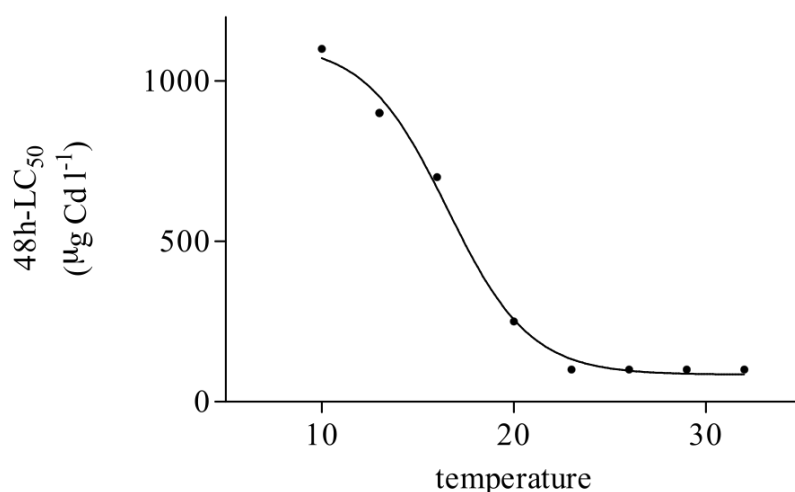


Figure 10. LC₅₀ values of *Daphnia magna* exposed to cadmium at temperatures 10-32°C. The regression line is a Boltzmann sigmodial found in GraphPad Prism 5. The dotted lines shows the 95% confidence bands of the curve. Best fit values and 95% confidence intervals are shown in table 5. Modified from Heugens et al. 2003.

Table 5. Best fit values and 95% confidence intervals for the Boltzmann sigmodial equations.

Test organism	Contaminant	Salinity ‰	Pmax	Pmin	V50	Slope	Reference
Best fit values (95% confidence intervals)							
<i>Artemia salina</i>	potassium dichromate	5	172.7 (2.3-343.1)	10.5 (-238.6-259.5)	21.6 (6.9-36.4)	1.9 (-9.7-13.5)	Persoone et.al. 1989
<i>Artemia salina</i>	potassium dichromate	20	167 (147.9-187.0)	6.6 (-20.8-34.1)	21.3 (19.7-22.9)	2.0 (0.5-3.4)	Persoone et.al. 1989
<i>Artemia salina</i>	potassium dichromate	35	271 (202.6-339.6)	16.7 (-23.6-57.0)	16.4 (14.2-22.9)	1.8 (0.03-3.7)	Persoone et.al. 1989
<i>Daphnia magna</i>	cadmium		1118 (954.1-1282)	84 (18.2-150.6)	16.6 (15.4-17.7)	2.2 (1.1-3.2)	Heugens et.al. 2003

Table 6. Best fit values and 95% confidence intervals for semilog line.

Test organism	Contaminant	Salinity ‰	Slope		Yintersept	Reference
			values (95% confidence intervals)			
<i>Artemia salina</i>	sodium laurylsulphate	5	-0.0293 (-0.0383-(-0.0202))		2.5 (2.4-2.6)	Persoone et.al. 1989
<i>Artemia salina</i>	sodium laurylsulphate	20	-0.0270 (-0.0345-(-0.0195))		2.2 (2.1-2.2)	Persoone et.al. 1989
<i>Artemia salina</i>	sodium laurylsulphate	35	-0.0270 (-0.0393-(-0.0146))		2.0 (1.8-2.2)	Persoone et.al. 1989

Bao et al. (2008) exposed the adult marine copepod *Tigriopus japonicus* to the antifouling biosides chlorothalonil and copper pyriithione (CuPT) at the temperatures 4, 10, 25, 35 and 40°C. The solvent controls started to die at 35°C and at 40°C there was 100% mortality for the solvent controls. At the lowest temperature examined 4°C there were no mortality at the controls. For each temperature 96 hours LC₁₀ were calculated. For both contaminants there was a trend to increase in toxicity at both the low and high temperatures examined (figure 11). Although it was only at 4°C there was a decrease in the low temperature range. Furthermore as the decrease was low compared to the other temperatures, the decrease could be the variance of the results. The non linear regression lines are second order polynomial fitted in GraphPad Prism 5. Values and 95% confidence intervals are shown in table 7.

Second order polynomial: $Y(x) = a \cdot bx \cdot bx^2$

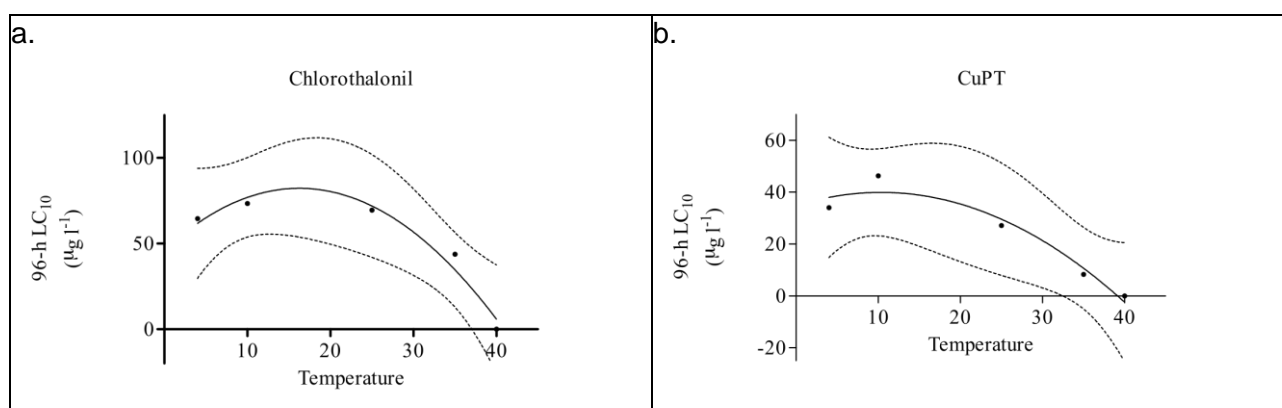


Figure 11. The adult copepod *Tigriopus japonicus* exposed to the antifouling biosides chlorothalonil and copper pyriithione (CuPT). Second order polynomial regression is used for the non linear regression. Best fit values and 95% confidence intervals are shown in table 7. Modified from Bao et al. (2008).

Table 7. Best fit values and 95% confidence intervals for second order polynomial line.

Test organism	Contaminant	a	b	c	Reference
		Best fit values (95% confidence intervals)			
<i>Tigriopus japonicus</i>	chlorothalonil	46.3 (-3.3-95.8)	4.4 (-1.6-10.5)	-0.146 (-0.272-0.000211)	Bao et.al. 2008
<i>Tigriopus japonicus</i>	copper pyriithione	34.7 (-1.1-70.6)	1.0 (-3.4-5.4)	-0.048 (-0.147-0.050)	Bao et.al. 2008

Saha and Kaviraj (2009) exposed the freshwater catfish *Heteropneustes fossilis* for 24 hours to 1 µg l⁻¹ of the pesticide cypermethrin at the temperatures 3-27°C and measured the % mortality (figure 12). There was no information about the mortality of the controls. In this experiment the

toxicity increased with temperature until the plateau where the toxicity stayed constant. The equation one phase decay found in GraphPad Prism 5 fitted best to the results.

One phase association: $Y = (Y_0 - \text{Plateau}) * \exp(-K * x) + \text{Plateau}$

Here, the plateau is defined as 100% mortality, Y_0 is when the line hit the y-axis and K is the rate constant. Best fit values and 95% confidence intervals are shown in table 8. These parameters have to be interpreted with caution as all points in the graph are based on approximations from the graph from the original paper (Saha and Kaviraj 2009).

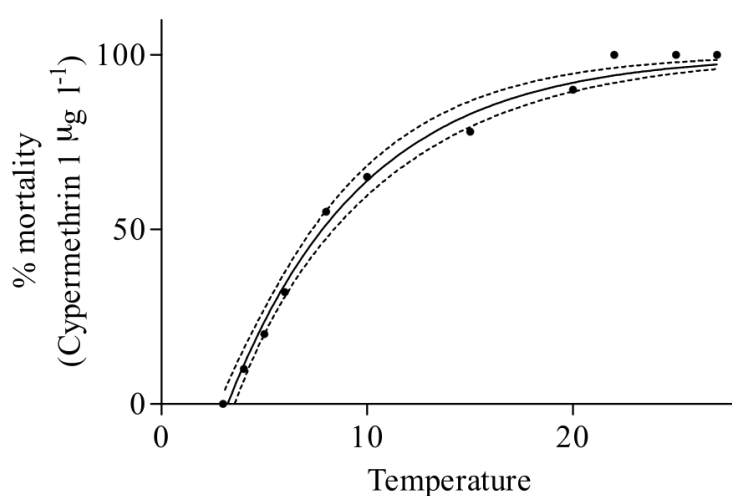


Figure 12. % mortality of *Heteropneustes fossilis* exposed to $1 \mu\text{g l}^{-1}$ of the pesticide cypermethrin for 24 hours at the temperatures 3-27°C. The fitted line is a one phase decay found in GraphPad Prism 5 and the dotted lines are the 95% confidence intervals. Best fit values and 95% confidence intervals are shown in table 8. Modified from Saha and Kaviraj (2009).

Table 8. Best fit values and 95% confidence intervals for the equation one phase decay found in GraphPad Prism 5.

Test organism	Contaminant	Y_0	K	Reference
Best fit values (95% confidence intervals)				
<i>Heteropneustes fossilis</i>	cypermethrin	-62.06 (-80.04- (-44.09))	0.150 (0.129-0.171)	Saha and Kaviraj 2009

3. Summary

This meta analyses has been performed in order to assess the potential for the development of model parameterizations from existing studies. In general few experiments have dealt with the issue of combined effects of contaminants and temperature, and not all contaminants studied are relevant for the marine environments. For example the pesticides cypermethrin and diazinon which degrade rapidly with a half life of days they are not expected to reach the marine waters (Moore et al. 2008, Saha and Kaviraj 2009). Furthermore for the purposes of modelling marine systems previous studies have primarily involved freshwater species. Adding to the complexity

species specific responses to contaminants are typical (e.g. McKim et al. 1978, Besser et al. 2005). Hence extrapolation from freshwater species to marine species although informative has the potential to produce results which are not based on a sound experimental basis.

To gain a better knowledge on the impact of contaminants on the marine environments clearly multi factorial experiments including a range of different temperatures it is necessary. Experiments should be chronic in nature with marine organisms exposed to relevant contaminants in a temperature range where both the temperature optimal and the extremes of high and low temperatures are included. Experiments have been performed under the MEECE project where these requirements have been fulfilled.

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