ITOPF R&D Award: A Shipping Industry’s Initiative to Support Research and Development

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Abstract

ITOPF’s shipowners and their Protection and Indemnity (P&I) insurers have established the annual ‘ITOPF R&D Award’ to encourage organisations worldwide to inspire innovative thinking and to present ideas that could provide solutions to some of the challenges faced in spill response and environmental monitoring. Each year £50,000 is made available to fund R&D projects that make a valuable contribution to improving the knowledge and understanding of issues related to accidental marine pollution. Since the creation of the Award in 2012, three projects have been initiated: FishHealth, SLAM and FAMERR.

FishHealth, is a 3-year project run by consortium of four research institutes led by LEMAR (Laboratoire des Sciences de L’Environnement Marin, France) that started in 2012. The project is a methodology for a physiology-based, ecologically relevant assessment of fish health, to provide information on the impact of chemically dispersed oil on marine fish.

Swansea Laboratory for Animal Movement (SLAM), based in Wales, is the beneficiary of the 2nd annual ITOPF R&D Award. The Award was used to fund a 1-year post-doctoral study to develop a novel ‘back-pack’ system to track rehabilitated oiled birds without compromising their wellbeing.

Finally, the beneficiary of the 3rd annual ITOPF R&D Award is the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in the UK. The project, called ‘Factors Affecting Marine Emergency and Response Research’ (FAMERR), aims to determine realistic spill profiles for two chemicals, aniline and butyl acrylate, so as to improve decision-making for spills in different geographic areas and seasons.

A review of two projects FishHealth and FAMERR, preliminary results and conclusions will be presented in the paper.

1. Introduction

ITOPF is recognized and respected globally as a source of objective technical expertise in the area of accidental spills of oil and chemicals from ships. Its mission is to promote effective response to marine spills of oil, chemicals and other substances. Investing in R&D is one way to enable this objective to be met.

Each year, over 7800 million tons of oil, chemicals, raw materials and finished goods are safely delivered by sea. Nevertheless, from time-to-time, accidents do occur that result in pollution of the marine environment by oil or chemicals. The severity of the pollution depends upon many factors. Continuous improvement in the understanding of the fate and effects of these substances will lead to improvements in accepted ‘best practice’ for spill response and environmental monitoring.

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The purpose of the initiative is to encourage organisations worldwide to inspire innovative thinking among their students and to present ideas that could provide realistic solutions to some of the challenges faced in spill response and environmental monitoring.

Each year, up to £50,000 is made available to fund R&D projects that make a valuable contribution to improving the knowledge and understanding of issues related to accidental marine pollution.

Since the creation of the Award in 2012, three projects have been initiated: FishHealth, SLAM and FAMERR.

2. FishHealth

In 2012, the 4-year project FishHealth, run by a consortium of four research institutes led by LEMAR (Laboratoire des Sciences de L'Environnement Marin, France) was the award recipient of the 2012 ITOPF R&D Award.

It addresses two areas of "fish health" where the results may have a direct input into the operational aspects of the response to an oil spill: Seafood quality in assessing the potential risks of tainting of fish stocks when dispersants are used and the impacts of dispersant use on fin fish populations by provide information on whether fish survival/growth of stocks exposed to dispersants might be impacted in the medium- and longer-term.

2.1 Introduction

The general aims of environmental monitoring and ecological risk assessment are to detect adverse impacts of toxicants upon organisms, and to predict their consequences upon population dynamics and production. During the last decade, substantial efforts have been devoted to develop and apply methodologies that evaluate anthropogenic impacts upon fish, and predict the consequences of exposure to chemical compounds. The initial objectives of this line of research were to generate early warning indicators that are triggered well before any measurable effects on individuals and populations, and which can assist in identifying the causes of these population effects (Forbes *et al.*, 2006).The proposed study investigates the impact of untreated and dispersant-treated oil upon fish response by applying the diagnostic tools commonly used to assess human health status using standardized challenges tests designed to assess individual incipient lethal oxygen saturation, incipient upper lethal temperature and critical swimming speed. Our reasoning was soundly based on the common and long-standing knowledge that these performance traits can be used as a bio-indicator of functional integrity and are useful markers of fish health (Tierney and Farrell, 2004; McKenzie *et al.,* 2007; Roze *et al.,* 2012; Claireaux *et al.,* 2013). The transfer of pre-exposed fish populations to a set of semi-natural tidal ponds, as a field study, evaluated the capacity of the proposed methodologies for providing a reliable diagnosis of fish health condition and a future prognosis for the population in an ecologically realistic setting (Claireaux *et al.,* 2013).

2.2 Methodology

2.2.1 Experimental Protocol

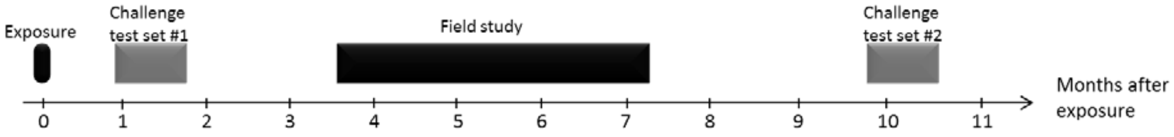

Figure 1: Schedule of the Experiment. The experiment began when fish has been exposed.

Experiment was conducted according to the schedule shown in figure 2-1. The fish used were 1-year old sea bass (*Dicentrarchus labrax*) measuring 11.27 ± 1.06 cm in length and weighing 17.74 ± 4.96 g. Using three environmental challenge tests, individuals' physiological ability for environmental adaptation was assessed a month after the exposure (challenge tests set #1, medium-term effects assessment). Then, fish were transported to tidal ponds for a 4-month period in order to validate the ecological relevance of the performances measured. Then, fish were bring back to the laboratory, kept in common garden for a 3-month period before the third challenge test set (long-term effects assessment) (Figure 1).

2.2.2 Exposure Condition

The study was conducted using weathered Arabian light crude oil. The dispersant used in the current study was the Corexit 9500, used in the BP Deepwater Horizon oil spill. Four experimental conditions were tested in triplicate (100 fish per replicate). One gram of dispersant (DISP), 5 g of oil (OIL) or both (OIL + DISP) was filled in each tank. Followed the weathering process, fish were randomly allocated in the 12 tanks (n = 100 / tank). 48 hours after the beginning of the exposure period, fish were recovered, briefly bathed in clean seawater before being return into their rearing tank. Control fish (CONT) followed the same procedure but were not exposed to the chemical compounds.

2.2.3 Fish Health Assessment

Similar to what is practiced in medicine, fish health was assessed using a set of challenge test. A challenge tests set included 3 physiologically based and ecological relevant challenge tests. The hypoxia challenge test (HCT), the temperature challenge test (TCT) and the swimming challenge test (SCT). HCT was conducted according to Claireaux *et al*. (2013), and consisted in a systematic decrease of water oxygenation. Individuals' incipient lethal oxygen saturation (ILOS) was determined which corresponded to the oxygen saturation where fish cannot maintain its equilibrium. Experiment ended when the last fish lost its ability to maintain its equilibrium. TCT consisted in a systematic increase of the water temperature (Claireaux *et al*., 2013). Critical temperature (Upper Incipient Lethal Temperature, UILT) was determined. As for ILOS, it corresponded to the amount of oxygen where the fish lost its ability to maintain its equilibrium. SCT consisted in the determination of the critical swimming speed of each fish from our population. For this purpose fish were placed by groups of 60 fish in a swimming chamber (Figure 2). The test consisted in progressively increasing the water velocity in the chamber (5 $cm.sec^{-1}$ every 10 min). As fish reached exhaustion and couldn't remove themselves from the grid placed downstream from the swim chamber, they were removed from the flume via a hole situated above the back grid. Fish were identified and the corresponding time and water speed was noted in order to calculate U_{crit} (Brett, 1964).

Figure 2: Swim Tunnel Used for the Swimming Challenge Tests. The swimming chamber is 200 ×20 cm and the maximum water velocity is 150 cm s^{-1}. Photos: Nicolas Le Bayon (Ifremer).

2.2.4 Field Experiment

Fish were transported to Littoral Environnement et Sociétés (LIENSs) field station in L'Houmeau, France (distance from Brest: 450 km). L'Houmeau mesocosmes' set up has been described in Claireaux *et al.* (2013). Fish were released into 7 ponds (25 fish per treatment per pond) on May 31st 2013 for six months. During that period ponds were drained twice to assess survivorship and growth (After a month and half (July 11th) and four month (September 9th)).

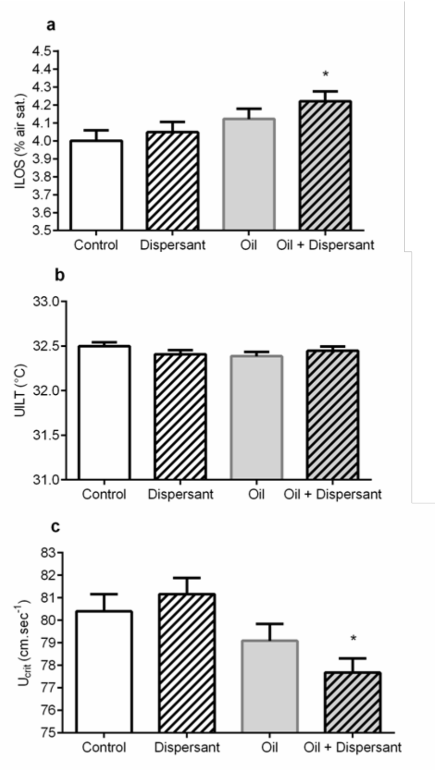

Figure 2: Mean of the Individual performances (a: ILOS, b: UILT, c: Ucrit) Observed During the Challenge Test Set #1. * indicates values significantly different from the control ($p < 0.05$)

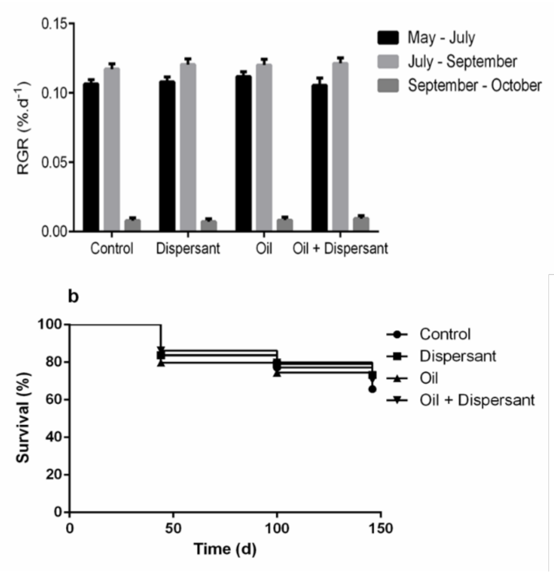

Figure 3: Growth (a) and Survival (b) Monitoring Throughout the Field Experiment According to Exposure Condition.

2.3 Results

2.3.1 Medium-term Effect of the Exposure

Figure 3a shows ILOS of fish exposed to the different conditions. ILOS of fish from the control group was close to 4 % air sat. Hypoxia tolerance of the same range was observed for the fish exposed to the dispersant or oil alone. However, fish exposed to the chemically dispersed oil had a significant higher ILOS (4.2 % air sat.). That means that chemically dispersed oil impact fish hypoxia tolerance. UILT of fish from the control group was close to 32.5 °C and didn't vary significantly whichever the exposure condition (Fig. 3b). Critical swimming speed of fish from the control group swam until ≈ 80 cm.sec^{-1} (Fig. 3c). Similar performances were observed for fish exposed to the dispersant or oil alone. However, fish exposed to the chemically dispersed oil swam significantly slower (≈ 78 cm.sec-1) than fish from the control group. Chemically dispersed oil had a significant impact on fish swimming performances.

2.3.2 Field Experiment

Figure 4a show relative growth rate (RGR) of fish exposed to the 4 different conditions. Summer (July to September) was associated with the best growth period. During autumn (September - October) fish growth was markedly decreased. However, no difference of RGR was observed between the exposure conditions whatever the period analyzed.

Survival analysis showed a survival rate higher than 60 % at the end of the field experiment (Fig. 4b). Moreover, mortality was regular throughout the experiment. This analysis also demonstrated that there was no difference of survival between the pond and the exposure condition.

2.3.3 Long-term Effect of the Exposure

Analyze of fish performance at the hypoxia challenge 4 months after the field experiment (11 months after the exposure) highlighted two main points (Fig. 2-5). First, ILOS of fish from the control group was close to 2 % air sat. Hypoxia tolerance of the same range was observed for the fish exposed to the dispersant, oil alone or chemically dispersed oil. UILT of fish from the control group was close to 28.2 °C and didn't vary significantly whichever the exposure condition. Critical swimming speed of fish from the control group was close to 60 cm.sec^{-1}. Similar performances were observed for fish exposed to the dispersant, oil alone or chemically dispersed oil. Exposure had no effect on fish performances 11 months after it has been conducted. Secondly, fish were more tolerant to the hypoxia, less tolerant to the heat and swam slower than during challenge test #1.

2.4 Conclusions

This study showed no medium-term effect of dispersant or oil alone. However a medium-term effect of exposure to chemically dispersed was observed on hypoxia tolerance and swimming performance. This result suggested that dispersed oil may cause more pronounced impacts in the short and medium term than oil or dispersant alone. Nevertheless, the field study demonstrated that under moderate environmental pressure this effect had no consequences on growth and survival. Moreover, no long-term effect of the exposure had been observed. This present study present preliminary results and it should be repeated in order to validate the results. Also, it could be judicious to test different concentrations of chemically dispersed oil to assess if there is a dose response effect. Additionally, it would be relevant to test if the consequences of a possible impairment of the performances under a stronger environmental pressure.

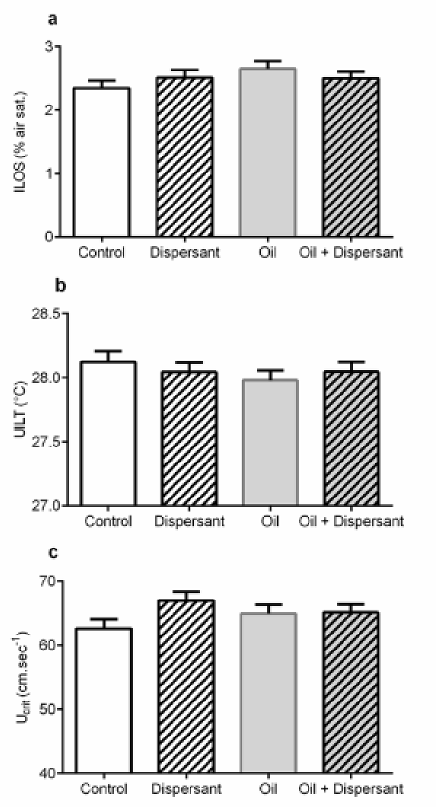

Figure 4: Mean of the Individual Performances (a: ILOS, b: UILT, c: Ucrit) Observed During the Challenge Test set #2. * indicates values significantly different from the control ($p < 0.05$)

3 FAMERR (Factors Affecting Marine Emergency and Response Research)

3.1 Introduction

It is recognised that hazardous and noxious substances (HNS) transported at sea could lead to a broad range of marine spill scenarios due to the variety of the cargos transported through national and international waters. The fate, behaviour and the impacts to marine organisms of spilled chemicals are dependent on the local marine conditions. While great efforts have been made to study the hydrological influences on the dispersion and movement of spilled HNS, little is known on the influences from other key marine features, e.g. water temperature, salinity. These factors influence the fate & behaviour and ecotoxicity of the HNS, and consequently, the decision-making on response and remediation options.

Under the European project ARCOPOLplus (Atlantic Regions Coastal Pollution Response), the toxicological risks of a few HNS have been assessed using selected seaweed species, tube worm, crustacean, larvae of sea urchin and juvenile fish (ARCOPOLplus Project report, 2013). In the continuation of this project, FAMERR studies the fate and ecotoxicity of a few selected chemicals, and their dependence on environmental parameters such as salinity and temperature.

Since the project commenced in June 2014, experiments have been carried out at 20°C which is the standard culture temperature for the selected species, but is also representative of summer temperatures of the sea surface water in coastal areas in the Southern North sea, the Channel and Bay of Biscay and 30°C, which represents the surface temperature near equator and in Persian Gulf. Salinities ranging between 30 ppt and 40 ppt have been applied, which are characteristic of coastal water influenced by estuarine inputs and waters with high salinity, e.g. part of Mediterranean Sea and Red Sea.

Aniline and zinc sulphate were the first two chemicals being tested in this project. Aniline is an important intermediate in a wide range of industry sectors and is shipped worldwide in high volume (Review of Maritime Transport, UN Conference on Trade and Development, 2011). Upon at sea release, it may sink slowly in water, and may be dangerous to aquatic life in high concentrations. The toxicity of aniline to marine organisms, particularly under different salinities and temperatures, has not been well studied. Therefore, aniline has been selected as one of the first test chemicals. In contrast, comprehensive studies have been carried out to study the toxicity of zinc (Park et al 2014, Zhou et al 2014). At the same time, zinc compounds are also transported in large quantities by sea (UN Conference on Trade and Development Report).

3.2 Methodology

3.2.1 Fate Simulation Using Chemical Model

A hydrodynamic transport model CHEMMAP (RPS/ASA) was used to predict the fate of chemicals (McCay et al., 2006). A near shore spill scenario (depth ~10m) with moderate (~ 0.3 m/s) tidal currents was used for the simulation. The influence of wind was not included in the simulation. This spill scenario represents a potential worst case scenario as any chemical spilled is likely to remain at incident location for a prolonged period than an offshore spill or release under higher tidal current / wind energy. Vertical mixing was set to a relatively high value ($Kv¬ = 0.1\ m^2\ s^{-1}$) for a well-mixed water column which was considered a representative worst case for the coastal location modeled and was the highest default value recommended in the CHEMMAP model. A surface spill of 1,000 tonnes of aniline over a four-hour period was used as the release scenario for the modelling. Typically, release over prolonged period, i.e. over four hours, would result in increased evaporation and enhanced dilution.

3.2.2 Toxicity Test

Constant exposures and time based exposures were performed at different temperatures (20 and 30 °C) and salinities (30, 35 and 40 ± 1 ppt). Toxicity test results were compared to evaluate the effect of these variables on the toxicity of different HNS.

3.2.2.1 Materials and Equipment

Aniline (ACS grade ≥99.5%), zinc sulphate (monohydrate, ≥99.9% trace metals basis) were prepared in filtered seawater. The effective concentration in stock solution was analysed using Gas Chromatography Mass Spectrometry (GCMS). This analysis has an assured detection limit of 2 µg/L. The concentration of zinc was analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Natural sand-filtered seawater is pumped from coastal waters adjacent to the laboratory through the Cefas seawater circulation system. Seawater used for the toxicity tests was subsequently filtered through a 0.2 µm filter and then sterilised using a UV filter to eliminate pathogens and parasites. Salinity was adjusted using TropicMarine synthetic salts (TropicMarine®, Wartenberg, Germany) to obtain the experiment salinity. Tests at 30°C were performed inside incubator chambers (INNOVA 44, New Brunswick Scientific U.K. LTD, St. Albans, UK). All the tests were carried out at a 16:8 (light: dark) cycle.

3.2.2.2 Test Species

Copepod Tisbe battagliai was chosen as the test species because of its sensitivity, ecological relevance and because of its use in previous projects. Copepods are small crustaceans that are frequently dominant secondary producers in marine zooplankton and are accordingly important to the marine food web. Copepods belonging to the genus *Tisbe*, which have been widely used in toxicity assessment because of their small size, short lifecycle and the ease of continuous culture (Hutchinson et al., 1999a and b).
The *Tisbe battagliai* used in this study were obtained from Guernsey Sea Farm and used within two weeks of delivery date. *Tisbes* were acclimatised in house for at least a week before the beginning of the test.

3.2.2.3 Standard Toxicity Test and Time-based Study

Standard 48 hour exposure studies were conducted with juvenile copepods. The concentrations chosen for aniline were 0, 0.3, 1, 3, 10 and 30 mg/L. Zinc concentrations (as zinc sulphate) used in the tests were 0, 0.1, 0.18, 0.32, 0.56, 1.0 and 1.8 mg/L. Observations of mortality and number of alive and dead juveniles were made at 24 and 48 hours. Water qualities were recorded at the beginning and at the end of each test. A parallel zinc reference test was carried out to ensure the batch of organisms tested were of similar sensitivity to previous batches. In both tests five juvenile *Tisbe* were placed in 5 ml test solutions in a 12 well, polythene plate. Four replicates were used per treatment and eight replicates in the control. Observations on copepod mortality in each test chamber were made using a binocular stereomicroscope (with dark field illumination and a magnification of 6–8 ×). Juvenile *Tisbe* were assessed as dead if, after 20s of gentle agitation of the copepod, no movement was observed.

3.3 Results and Discussion

3.3.1 Fate Modelling of the Influence of Temperature and Salinity

The influences of temperature and salinity on the fate of aniline as modelled in CHEMMAP are presented in Figure 6 and Figure 7. From the simulation results on aniline, it is clear that temperature has noticeable effect on the amount of chemical that may evaporate and degradation. As a result of enhanced evaporation and degradation at 30°C, the amount of aniline remains in water column after 24 hours is 30% less than the spill scenario at 10°C. In addition, regions with high water temperatures are likely to have water column stratification, which is not taken into consideration in this simulation. For surface spills, such stratification would inhibit the free movement to the seabed, and potentially further enhance the surface evaporation. Both of these effects might lead to reduced benthic exposure in turn. However, the exact extent of the temperature effect depends on the properties of the chemical, e.g. whether it evaporates and degrades readily.

The influence of salinity is more complicated, and is dependent on the properties of individual chemicals. As shown in Figure 7, in the case of aniline, increased salinity would cause a marginal increase in evaporation, and consequently, a minor reduction of the remaining amount in water column. It is worth pointing out that the effects of salinity depend on the physical behaviours of individual chemicals in different density water. Therefore, the influence of salinity is chemical-specific, and cannot be generalised.

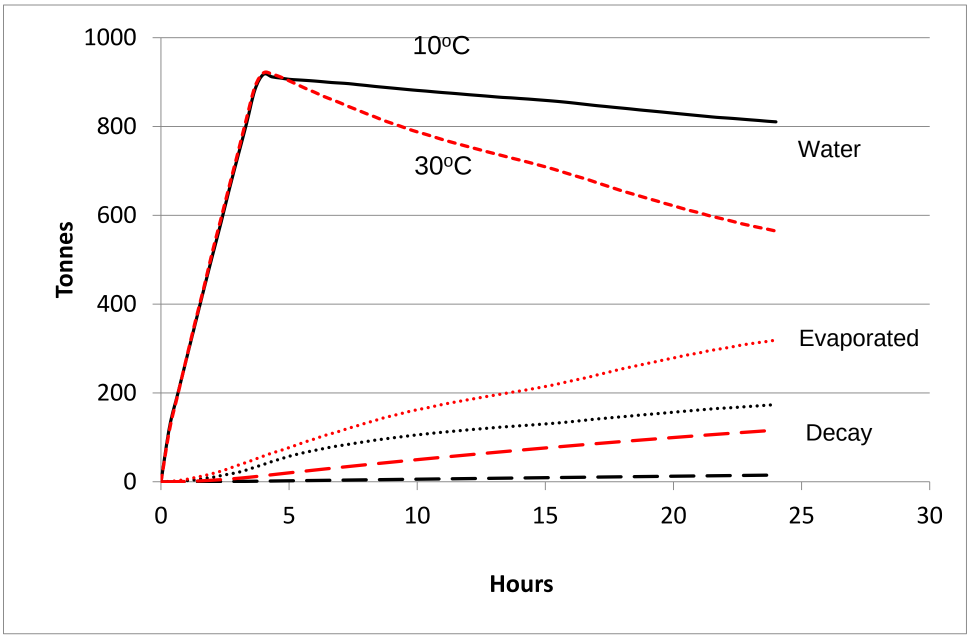

Figure 6: The Influence of Aqueous Temperature (black 10°C and red 30°C) on the Fate of Aniline (at salinity 32 ppt). Bold lines are water concentration, dots evaporation, dashes degradation

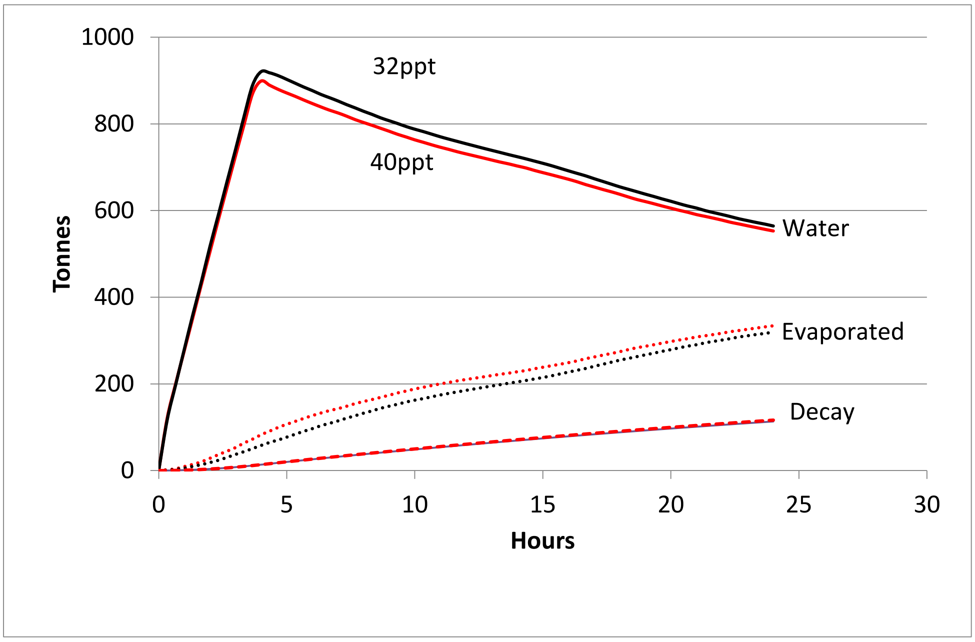

Figure 7: The Influence of Salinity on the Fate of Aniline (at 30°C). Black lines 32ppt salinity and red lines 40ppt salinity. Bold lines are water concentration, dots evaporation, dashes degradation

3.3.2 Effects of Salinity on Toxicity

The LC50 exposure results using test species *Tisbe battagliai* at different salinities are presented in Figure 8 (aniline) and Figure 9 (zinc).The preliminary results suggest that zinc exhibits higher toxicity at low salinity (i.e. 30 ppt) relative to at intermediate (35 ppt) salinity and high (40 ppt) salinity. Aniline in contrast was least toxic to *Tisbe battaglai* at an intermediate salinity of 35 ppt but had a similarly high toxicity at 30 and 40 ppt. Additional aniline toxicity tests on *Corophium volutator*, a species less sensitive to the compound than

Tisbe battagliai, in contrast to Tisbe, showed a decrease of sensitivity at both 35 and 40 ppt salinity relative to at 30ppt salinity (data not shown).

These results are consistent with some of the findings published in the literature. In a recent study evaluating the effects of salinity on acute toxicity of copper and zinc on copepod Tigriopus japonicas, it was suggested that the decrease of toxicity at increased salinity was attributed to the decrease of dissolved (zinc and copper) ions in solution (Park et al, 2014). In the present study analysis of the primary stock solutions did not indicate major differences in dissolved zinc concentration but it is possible that on make up of test solutions some precipitation of zinc occurred thus reducing the dissolved concentration at the higher salinities. Precipitation of zinc at higher salinities has been reported in previous studies (Frost and Hales, 2007). The resulting lower toxicity (i.e. higher EC50 values) 0.32 mg/L at 40 ppt, 0.29 mg/L at 35 ppt, reducing to 0.15 mg/L at 30 ppt may be explained by a potential reduction in dissolved concentration with increased salinity. The toxicity data for aniline suggests that species related differences have contributed to the results for which Tisbe was less sensitive at intermediate salinity (35 ppt) compared to Corophium which was least sensitive at both salinities tested that were higher than 30 ppt. studies by Miliou, 1993 show that lifespan of a related Tisbe species is optimal at salinity of 38 ppt and declines either side of this value. In the present study the combined stress of salinity outside an optimal range may therefore have led to the observed results for Tisbe. Data for Corophium suggests this species is relatively tolerant of salinity concentrations across a wide range 2 – 50 ppt (McClusky, 1967) but in addition the higher salinity must also either limit aniline uptake by making it less bioavailable or reduces its uptake or increases its excretion thus reducing its toxicity.

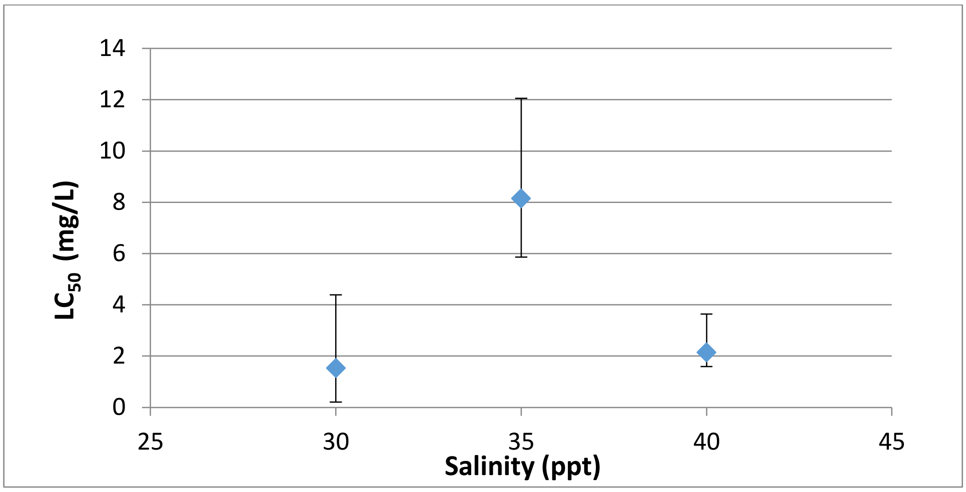

Figure 8: LC_{50} Calculated for the Different Concentrations Tested of Aniline at Three Different Salinities at 20 and 30°C during a 48 hours Constant Exposure of *Tisbe battaglai*. Error bars show upper and lower confidence limits.

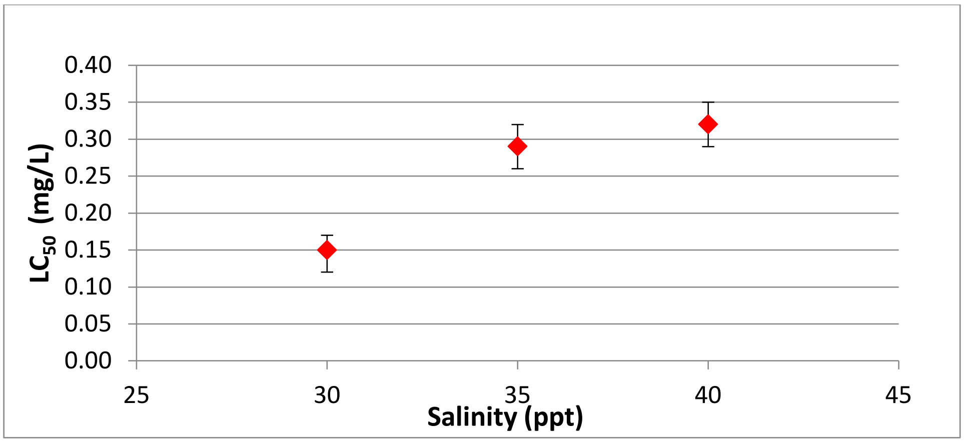

Figure 9: LC_{50} Calculated for the Different Concentrations Tested of Zinc Sulphate at Three Different Salinities at 20 °C for *Tisbe battaglai* During 48 hours Constant Exposures. Error bars show upper and lower confidence limits.

3.3.3 Effects of Temperature on Toxicity

Preliminary temperature related toxicity tests using *Tisbe battagliai* indicated that increased toxicity occurred with the increase of aqueous temperature but high control mortality confounded firm conclusions being drawn from the dataset so the data are not shown. However temperature is considered to be one of the most important factors influencing toxicity of chemicals (Heugens et al., 2001). The increase of temperature would typically lead to the increase of uptake rate among marine organisms (Heugens et al. 2003). Similar temperature effects have been published in literature from toxicity tests on *Daphnia* (Heugens et al. 2003) and *Tigriopus japonicas* (Kwok and Leung, 2005). A recent review on the toxicity of zinc, chromium and cadmium suggest that there is evidence for two different patterns of response for marine organisms exposed to metals at different temperatures: (i) Toxicity either increased at lower and higher temperatures around a given value (possibly as a result of the combined effect of temperature stress and toxicity on the organism when at non optimal temperature) or (ii) toxicity steadily increased as temperature increased probably as a result of increasing uptake of the toxicant (Zhou et al, 2014).

3.4 Conclusions

The preliminary results from this study seem to support the hypothesis that the selected HNS (i.e. zinc and aniline) have the highest toxicity on test species (i.e. *Tisbe battagliai)* at salinity of 30 ppt. However salinities >30 ppt whilst toxicity for some substances may be reduced due to reduced solubility (i.e. zinc) the pattern of toxicity for other substances where solubility is not affected may be less clear cut as the salinity tolerance of the test species may also contribute to increased sensitivity at salinities either side of an optimum. Although higher aqueous temperature is generally expected to enhance evaporation and degradation, as suggested by the modelling results, the preliminary toxicity test results here but more so data from the literature suggest that toxicity is increased at higher test temperatures. However in an environmental context it may be that over longer exposure periods the loss of aniline by evaporation and degradation will play an important part in reducing chronic toxicity. Work is continuing to investigate the effects of temperature and salinity on the behaviour and toxicity of a wider range of chemicals. Studies will also include other test species, e.g. seaweed, to determine the sensitivity at different trophic levels.

4 Acknowledgments

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