FishHealth, The 2012 R&D Award Winner

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

1) WHO ARE THE ORGANISATIONS?

LEMAR (Laboratoire des sciences de l’Environnement MARin) is a research unit involving 4 major French scientific institutions: CNRS, Ifremer, IDR and UBO. Research conducted by the LEMAR Marine Environmental Sciences Laboratory aims to increase the current understanding of the mechanisms that underpin the functioning of marine ecosystems and determine their resilience when submitted to anthropogenic influences.

CEDRE is a non-profit-making association in charge of improving spill response preparedness and strengthening the national response organization. It is responsible, on a national level, for documentation, research and experimentation on pollutants, their effects and the response means and tools that can be used to combat them. Its facilities include a greenhouse for toxicity studies and impact assessment of various pollutants on marine organisms, and a laboratory fully dedicated to pollutant characterisation using modern analytic equipment.

CNR-IAMC Oristano is a research unit that is part of a large marine institute involving 7 other units throughout Italy. Research carried out at CNR-IAMC Oristano includes ecophysiology, benthic ecology, ecological modelling, oceanography, marine geology. The activity of the Ecophysiology group focuses on the effect of global change (hypoxia, temperature, acidification) on fish behaviour and energetics.

The Zoology Department at UBC is home to the world’s strongest group of comparative animal physiologists, amongst whom fish physiologists are extremely well represented.

2) PROJECT DURATION AND TIMING

The project receives funding from ITOPF for 3 years, between June 2012 and May 2015.

From December 2013 to November 2014, the PhD student working on the project also receives funding from Total Fluides.

3) WHY DID ITOPF DECIDE TO FUND THIS RESEARCH?

As recent events continue to show, resorting to chemical dispersants to deal with oil spills in coastal environments is a real but controversial matter. At the centre of this controversy is whether the risk of ecological effects on marine species from toxic oil components increases or decreases when chemical dispersants are used. In this regard, two main issues must be addressed, one relating to the chemistry of the interaction between oil and dispersant, which has huge implications for
bioavailability, and the other relating to potential effects of the oil, the dispersant and the mixture upon the health status of living organisms.

To address these issues, the proposed research will:

- Validate a methodology to assess the health status of a fish population;
- Determine the threshold for fish avoidance reactions to water soluble fraction (WSF) of oil and to suspended oil droplets due to dispersion treatment;
- Provide operational information for the use of dispersant in coastal waters.

From ITOPF’s perspective, the project 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:

i) Seafood quality – assist in assessing the potential risks of tainting of fish stocks when dispersants are used.

ii) The impacts of dispersant use on fin fish populations – provide information on whether fish survival/growth of stocks exposed to dispersants might be impacted in the medium- and longer-term.

Both these areas have obvious economic considerations when managing fish stocks during a spill (e.g. harvesting bans) and on the potential claims for compensation for longer term reduced fish ‘health’ (e.g. reduced reproductive capacity, increased mortality etc.) Linked closely with these points are the operational considerations whereby questions regarding whether or not to use dispersants (due to concerns for the local fish stocks) can be evaluated alongside scientific data to support the advice given.

4) PRESENTATION OF THE FISH HEALTH ASSESSMENT TESTS

Measuring fish health: physiological bases and ecological relevance
During the last decade, substantial efforts have been devoted to developing and applying methodologies that evaluate anthropogenic impacts upon fish, and predict the consequences of exposure to chemical compounds. This study investigates the impact of untreated and dispersant-treated oil upon fish response to standardised tolerance tests designed to assess incipient lower lethal oxygen level, incipient upper lethal temperature and critical swimming speed, considering these performance traits as possible bio-markers of fish health.

Avoidance reaction
To fully assess fish’s adaptive repertoire when faced with a contaminated water body, the research will investigate the behavioural dimension of fish response to an oil slick, whether it is chemically treated or not. Fish detection ability and limits using classical behavioural indices of discomfort to increasing doses of oil droplets will be examined i.e., avoidance reaction and changes in distribution pattern.

Chemistry of oil-dispersant interaction:
The research is performed using crude Arabian light, which is well documented with regard to its physical and chemical properties. To take into account the changes that affect oil at sea, a weathering process is applied prior to dispersant treatment, simulating a 12 hour ageing of a slick at sea. To benefit
from ongoing studies, the dispersant tested is Corexit 9500 (Nalco), the main dispersant used in the DEEPWATER HORIZON incident.

5) VALIDATION AND INTERPRETATION OF THE CHALLENGE TESTS

The first phase of the work was conducted on an initial fish population of nearly 1200 individuals. During the first semester of the project, 600, 1-year old tagged seabass (Dicentrarchus labrax) were submitted to the 3 tolerance tests (hypoxia, heat and swimming). The temporal stability (repeatability) of individuals’ performance as well as the ability of the fish to recover from exercising maximally were tested and a database was created to contain, for each fish, the relevant biometric information as well as results at the various challenge tests.

Hypoxia challenge test (HCT)
HCT consisted of a rapid decrease in water oxygenation, down to 20 % air saturation in about one hour, followed by a much slower descent.

As soon as a fish lost its ability to maintain balance i.e., when the incipient lethal level (ILOS) was reached, it was removed from the experimental arena (Fig.1a), identified (pit tag reading; Fig.1b) and placed in a fully aerated recovery tank. The corresponding time and oxygenation level was also recorded (Fig.1c and Fig.1d). Less than 1 % mortality was observed following HCT.

Temperature challenge test (TCT)
TCT consisted of a period of rapid temperature increase (from temperature of acclimation to 27 °C in about 2.5 hours) followed by a slower increase (approximately 0.5 °C per hour) until the experiment ended. As fish lost equilibrium, they were removed from the tank, identified and placed in a recovery tank at their original acclimation temperature. The corresponding time and temperature (upper incipient lethal temperature, UILT) was also recorded. As for HCT, less than 1 % mortality was recorded in the days that followed TCT.
Swimming challenge tests (SCT)
The swimming flume consisted of two swimming chambers (2 × 0.2 m) in which the speed of the water is controlled using a pump and a frequency regulator. Groups of 60 fish were sequentially introduced in each chamber. A set of valves allowed the researcher to exercise one experimental group while the second group habituated to the swimming chamber (2h). The test involved 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, identified and the corresponding time and water speed was noted.

Swim tunnel used for the swimming challenge tests. Photos: Nicolas Le Bayon (Ifremer).

RESULTS

At the end of the first year, two initial objectives of the research have been addressed i.e.
- 1) validating protocols and improving interpretation of individuals’ performance at the challenge tests and
- 2) investigating the ecological-relevance of chemical exposure-related loss in performance at the challenge tests.

1) Validation of the protocols

Although water temperature and fish size changed over the course of the experiment, individuals’ performance measured during the challenge tests were found temporally stable (repeatable) even when challenge tests were repeated 8 months apart (Fig.5). This long-term repeatability is remarkable for hypoxia tolerance (Fig.5a) and swimming speed (Fig.5c) as this has never been reported before. This result also confirms the view that these environmental adaptation performance traits are potential indicators of fish health and vulnerability under natural conditions.
Although statistically significant, repeatability of heat tolerance is less convincing (Fig.5b) and will require further investigation. It is quite likely that this performance trait is more sensitive to the initial (acclimation) thermal condition.

2) First experimental exposure

The principal goal of this experiment was to monitor, and compare, fish performance at the tolerance tests before and after they had been exposed to either mechanically- or chemically-dispersed oil. During the first semester of the project it was decided that one nominal concentration for both chemically and mechanically dispersed treatment (24h-weathered BAL : 25 g; Corexit: 1 g) and an exposure duration of 48 hours would be tested during this first series of experiment.

Fish exposure was conducted in a set of 12 polyethylene tanks (300 l; 100 fish per tank; Fig.7). These tanks were equipped with a homemade mixing device (Fig.7b) comprising a funnel and a submersible bilge pump (Johnson; L450 - 500GPH - 12V) which ensured that the surface water and floating oil would be homogenised.
Four experimental conditions were tested in triplicate i.e., control, dispersant alone (Corexit: 1 g), oil alone (BAL: 25 g) and oil + dispersant (BAL: 25 g; Corexit: 1 g). Water sample (100 ml; triplicate) were taken before as well as 4 h, 24 h and 48 h after fish had been introduced in the tanks.

Forty eight hours after they had been removed from the exposure tanks, 9 fish per experimental condition were euthanized and their white muscle, liver, gale bladder and heart sampled were analysed. The following results only concern the HAP level present in the white muscles.

**PAH concentration**

PAH concentration in the white muscles of fish from the control and dispersant-alone groups were below the quantification limits at all time (1 ng g$^{-1}$; Fig.9). On the other hand, at 2 days post exposure, white muscles HAP concentration in fish from the oil-alone and oil + dispersant groups were approximately 300 times higher. However, 29 days later, these concentrations had returned below the quantification limit.

Comparison of fish performance at the hypoxia challenge test before and after exposure highlighted two main points (Fig.10).

i) As illustrated in Fig.10a, a general loss of tolerance to reduced oxygen availability was observed in the control group. This right shift of ILOS shift was not unexpected and is attributable to the temperature driven increase in fish metabolic rate and connected oxygen demand. Between 15 °C and 18 °C, sea bass metabolic rate is indeed multiplied by nearly 5 for every 10 °C increase in acclimation temperature (Claireaux and Lagardère 1999). This temperature-driven loss in hypoxia tolerance was observed in all experimental groups but...
significantly aggravated in the oil + dispersant group.

ii) As observed for hypoxia tolerance, a shift in the incipient upper lethal temperature (IULT) measured before and after the exposure was observed in all the experimental groups. Again, this improvement was not unexpected and is also to be related to the change in acclimation temperature observed between the two series of challenges. Cold acclimated fish are classically reported to be less tolerant to heat than warm acclimated fish. However, although a trend may be noticed in the data (Fig. 11a), the amplitude of this shift was not statistically different from that observed in the control group.

iii) A similar pattern was also observed with regard to critical swimming speed ($U_{\text{crit}}$). Fish from the control group swim faster during the second test when compared to the first test (Fig. 12a). This improved swimming capability is mostly attributable to the rising acclimation temperature and resulting increased metabolic performance (Claireaux et al. 2006). However, it can also be ascribed, at least partly, to the increased fish size (11.4 ±0.1 cm to 12.2 ±0.4 cm). An improvement of similar amplitude in fish swimming capacities was observed in the dispersant group (Fig. 12b). However, the amplitude of the shift was significantly reduced in the oil-alone and oil + dispersant groups suggesting that these fish did not profit to the same extent from the seasonal warming of the water.

3) **Preliminary conclusions**

Data available to date suggest that oil exposure affects the response of fish to our hypoxia and swimming challenge tests. Moreover, impairment of fish performance was more marked in fish
exposed to a mixture of oil and dispersant. On the other hand, following contaminant exposure, fish responses to the temperature challenge test were not different among the experimental groups.

The initial hypothesis when designing the experimental protocol was that time was an essential element to consider in looking for impairments of such complex and integrated performance traits such as swimming capabilities, hypoxia tolerance and thermal sensitivity. Approximately 6 weeks following exposures, impairments in fish performance were noticed. However, we must ensure that these dysfunctions are maintained over time before they can be ascribed with any ecological relevance. To ascertain this point, 400 fish from our original population will be kept in our laboratory rearing facilities to be challenged again 3-4 times during the coming year. The rest of the population (n = 700) was transferred to a set of semi-natural tidal earthen ponds for our first field experiment.

**FIRST FIELD EXPERIMENT**

![Image of the L’Houmeau field site, showing the 10 parallel experimental earthen ponds, fish transfer, identification, and allocation, and an operational pond covered with a bird net.](image)

The first field experiment is currently under way. Fully identified fish from all four experimental groups (control, dispersant alone, oil alone and oil + dispersant) were transferred into L’Houmeau pond field site at the end of May 2013 (Fig.13). Sea water in these ponds is renewed at every tide and previous experiments and empirical observations suggest that food supply in each pond is sufficient to sustain 2 kg of fish.