Relevance and suitability of invertebrates swimming behavior as sub-lethal endpoint to be considered for ecotoxicological investigation

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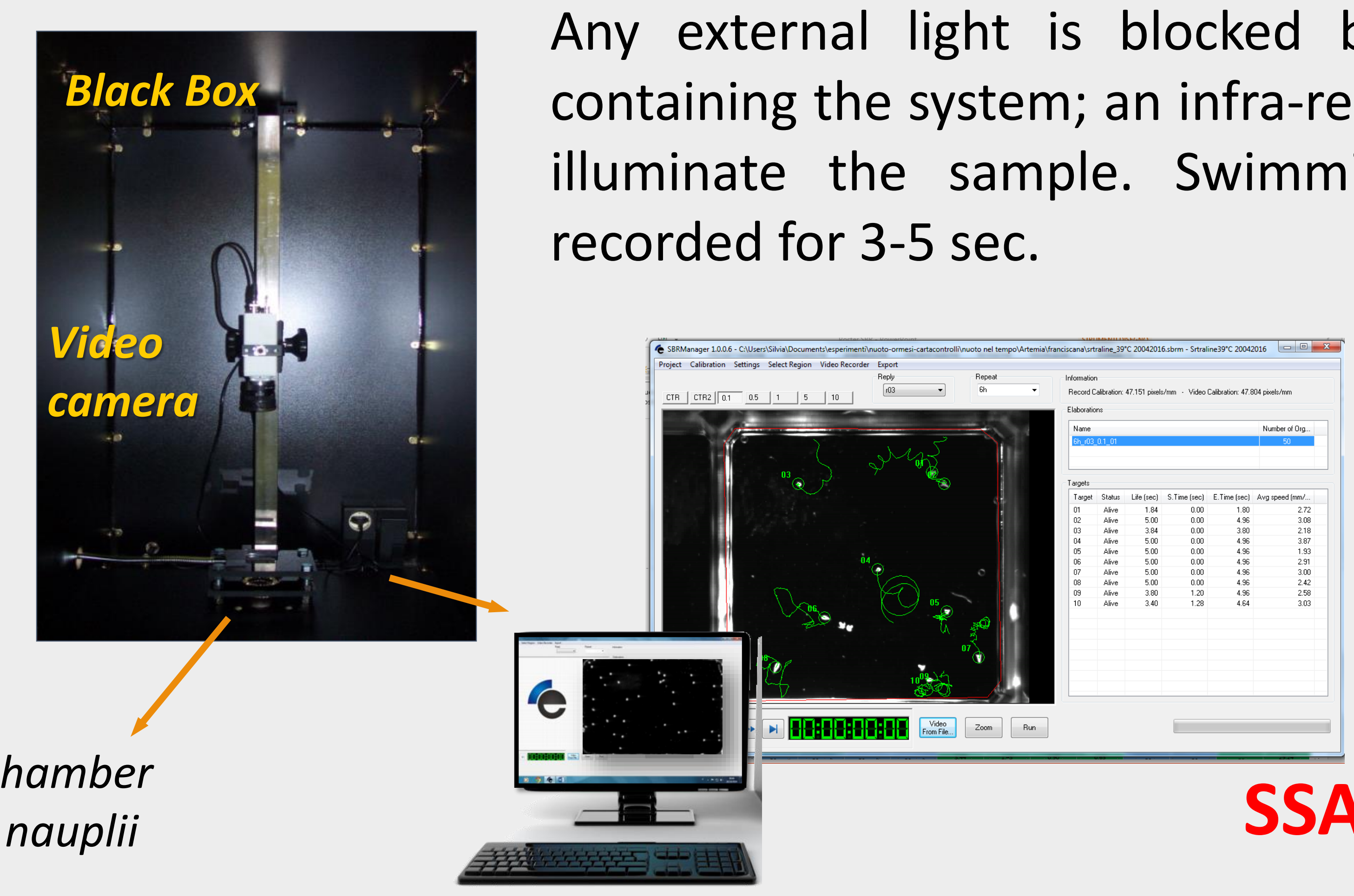
Synopsis: a selection of EC/LC_{50} values calculated exposing three marine invertebrates to different compounds are herein listed, demonstrating the sensitivity and relevance of the behavioral endpoint compared to the mortality one.

A video tracking recording system, namely Swimming Behavioral Recorder (SBR) system, has been developed at the laboratory of ISMAR-CNR (Genoa, Italy) to evaluate the naupliar swimming behavior of different marine invertebrates exposed to toxicants (Gambardella et al. 2017; Faimali et al. 2017). In years of researches, a consistent amount of data has been produced that support the relevance and the suitability of this methodology to be applied to aquatic invertebrates.

Swimming Behavioral Recorder system

A macro-objective records the paths of swimming larvae (*Artemia* sp.; *Amphibalanus amphitrite*; *Brachionus plicatilis)* kept in a *recording chamber* (21x34x3mm²). To minimize the vertical distribution, nauplii are maintained in 1 mL of volume.



Recording chamber containing nauplii

Any external light is blocked by a **black box** containing the system; an infra-red light is used to illuminate the sample. Swimming behavior is recorded for 3-5 sec.


Artemia sp.


A.amphitrite


B.plicatilis

Images are analyzed through an image processing software (SBR manager, by e-magine IT, Genoa, Italy) to obtain the average swimming speed for each sample (10-15 organisms).

$$SSA\ (\%) = 100 * (S_{control} – S_{treated})- S_{control}$$

Results

Artemia sp.

67% of cases, SSA test more sensitive than mortality test (LC_{50} >EC_{50})

	LC_{50} 24 h	EC_{50} 24 h	LC_{50} 48h	EC_{50} 48h
$K_2Cr_2O_7$	34.78 mg/L (30.36-39.84)	23.69 mg/L (21.33 - 26.31)	6.01 mg/L (5.08-7.11)	6.16 mg/L (5.44-6.97)
$CdCl_2$	* n.c	* n.c	* n.c	* n.c
ZnPt	* n.c	15.09 mg/L (8.53-26.70)	41.80 mg/L	7.33 mg/L (5.48-9.81)
MT-200	7.45 mg/L	7.43 mg/L (6.68-8.27)	7.39 mg/L	4.54 mg/L (4.04-5.11)
Eserina	* n.c	28.07 mg/L (13.81-57.08)	* n.c	8.83 mg/L (5.87-13.28)
Pirene	* n.c	* n.c	* n.c	0.71 mg/L (0.6-0.83)
Elutriates	* n.c	78.23 % (59.57-102.73)	* n.c	68.25 % (59.28-78.57)
Polystyrene 0.1µm	* n.c	* n.c	70.06 mg/L (49.57-99.01)	51.89 mg/L (39.78-67.69)
NanoSilver	* n.c	21.83 mg/L (13.67-34.86)	* n.c	20.33 mg/L (12.54-32.95)

B. plicatilis

64% of cases, SSA test more sensitive than mortality test (LC_{50} >EC_{50})

	LC_{50} 24 h	EC_{50} 24 h	LC_{50} 48h	EC_{50} 48h
$K_2Cr_2O_7$	296,89 mg/L (276.62-318.64)	259 mg/L (229.77-293.03)	158,11 mg/L (141.33-176.89)	103,81 mg/L (86.78-124.19)
$CdCl_2$	* n.c	* n.c	* n.c	29.24 mg/L (7.74-110.45)
ZnPt	0.07 mg/L (0.06-0.07)	<0.005 mg/L	0.02 mg/L (0.02-0.03)	<0.005 mg/L
MT-200	0.54 mg/L (0.27-1.07)	0.01 mg/L (n.c.)	0.51 mg/L (n.c.)	0.02 mg/L (0.01-0.02)
Eserina	* n.c	45.62 mg/L (32.83-63.40)	* n.c	9.3 mg/L (n.c.)
Elutriates	* n.c	* n.c	* n.c	91.85 % (87.64-95.27)
Polystyrene 0.1µm	* n.c	* n.c	* n.c	* n.c

A. amphitrite

78% of cases, SSA test more sensitive than mortality test (LC_{50} >EC_{50})

	LC_{50} 24 h	EC_{50} 24 h	LC_{50} 48h	EC_{50} 48h
$CdCl_2$	3.44 mg/L (3.06-3.86)	1.43 mg/L (1.25-1.62)	0.90 mg/L (0.79-1.02)	0.65 mg/L (0.58-0.74)
$ZnCl_2$	3.10 mg/L (2.93-3.27)	1.93 mg/L (1.80-2.08)	1.66 mg/L (1.54-1.78)	<1 mg/L
ZnPt	* n.c	0.07 mg/L (0.06-0.09)	*	*
MT-200	2.24 mg/L (nc)	1.80 mg/L (1.63-1.98)	2.14 mg/L (2.04-2.23)	1.60 mg/L (1.30-1.96)
Eserina	* n.c	0.23 mg/L (0.19-0.28)	*	*
Chlorpyrifos	* n.c	0.10 µg/L (0.07-0.15)	0.30 µg/L (0.19-0.47)	0.05 µg/L (0.04-0.08)
Pirene	* n.c	* n.c	* n.c	* n.c
Elutriates	* n.c	46.44 % (36.47-59.13)	>100 %	35.38 % (28.41-44.05)
Polystyrene 0.1µm	* n.c	* n.c	* n.c	* n.c
NanoSilver	1.08 mg/L (1.04-1.13)	0.70 mg/L (0.62-0.80)	0.51 mg/L (0.48-0.54)	0.30 mg/L (0.25-0.36)

* n.c. not calculable at the maximum concetration tested

** missing data

🟥 EC_{50} >LC_{50} 🟩 LC_{50} >EC_{50} 🟦 EC_{50} =LC_{50}

- ✓ In 100% of cases, the swimming speed alteration (SSA) test is more or at least as sensitive than the mortality test
- ✓ SSA test sensitivity is particularly evident for nauplii of *A. amphitrite*

Conclusion

SBR system has proved to be a valid video tracking analysis method for ecotoxicological research that can be applied to different aquatic organisms. The swimming speed alteration demonstrated to be a sensitive endpoint to assess the likely damage exerted by chemical pollution and other stressors at very low concentrations, suggesting its possible use in environmental regulation.

Faimali, M., Gambardella, C., Costa, E., Piazza, V., Morgana, S., Estévez-Calvar, N., Garaventa, F., 2017. Old model organisms and new behavioral end-points: swimming alteration as an ecotoxicological response. Mar. Environ. Res. 128, 36–45

Gambardella, C., Morgana, S., Ferrando, S., Bramini, M., Piazza, V., Costa, E., Garaventa, F., Faimali, M., 2017. Effects of polystyrene microbeads in marine planktonic crustaceans. Ecotoxicol. Environ. Res. 145, 250–257.