SENSITIVITY OF MACROINVERTEBRATES TO SUBSTRATE BORNE VIBRATION

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ABSTRACT

To assess and understand the impact of anthropogenic vibration upon marine organisms we first need data on the detection capabilities of these species. Many marine developments involve techniques directly contacting the seabed, such as pile driving, and it is likely that benthic animals are being exposed to strong seabed vibrations during construction. There are few data available on their sensitivity to such stimuli. Vibration is likely to be detected by most marine invertebrates, and exposure to vibration may affect key behaviors such as the ability to escape predators and to forage successfully, but there has been little research in this field in recent years. Here, the sensitivity of macroinvertebrates (bivalves and crustaceans) to substrate-borne vibration was determined during vibration exposures. We investigated thresholds of unconditioned behavioral responses to a range of frequencies (5 – 410 Hz) under controlled conditions. Sensitivities and types of behavioral responses were related to measurements of seabed vibration taken in the vicinity of anthropogenic operations to determine critical exposure levels of macroinvertebrates. The data showed a high sensitivity of invertebrates to substrate vibration in a broad range of frequencies. The responses indicate that organisms are clearly affected by anthropogenic noise related to marine operations.

1 INTRODUCTION

Anthropogenic activities directly contacting the seabed, for example pile driving, drilling and dredging, produce vibrations which may travel great distances from source[1] for example as shear waves, surface waves or compressional waves[2,3]. However, whilst recent governance initiatives have focused upon marine underwater noise, the inclusion of seabed energy (substrate-borne particle motion) within this is unclear. Further accentuating the issue is that the abilities of benthic and demersal marine organisms to detect vibration via the substrate are largely undescribed, with much of the data being made up of anecdotal observations after exposures to unquantified vibration; or focussed upon non-marine species[6,7], with the topic only recently gaining interest in underwater noise review papers[8], as reviewed previously[4,5].

In crustaceans and bivalves, detection of vibration is likely to involve a variety of external mechanoreceptors upon the body surface and an internal statocyst system[9-14], yet data is largely restricted to semi-terrestrial or freshwater species rather than marine[12,15], as reviewed by other works[16]. In bivalves specific receptors are relatively undescribed[17-19]. For both groups the extent of sensitivity to vibration for marine species is not well understood. There is therefore a need for investigating the extent of sensitivity to vibration, the receptors and pathways used for the detection, and the effects of short term and continuous vibration upon marine organisms.

Here, bivalves and crustaceans were exposed to substrate-borne vibration within fully controlled laboratory conditions in order to assess their sensitivity in relation to anthropogenically-generated seabed vibration.

2 SENSITIVITY OF BIVALVES AND CRUSTACEANS TO VIBRATION

2.1 Methodology

Full experimental methods are described in recent work, currently in press[5, 20]. Two separate experimental periods were undertaken with hermit crabs (*Pagurus bernhardus*) and mussels (*Mytilus edulis*) using the same methodology and setup. The experimental tank (400 x 600 mm), was mounted upon a custom-made base, built to minimize external vibrations entering the tank. Animals were acclimatized overnight in the tank prior to experiments, and were free to move within an 'arena' (100 x 100 mm) at one end of the tank. At the other end of the tank, a stinger rod descended vertically to the sandy substratum, connected to an electromagnetic shaker situated above the tank. The shaker was mounted on a separate frame to the base. Sinusoidal signals of 7 frequencies (spanning 5 – 410 Hz, 8 second duration with 1 s rise and decay time), 11 amplitudes, greatest in the vertical plane, were presented to animals via the shaker and associated amplifier. The order of frequency presentation was fully randomized.

Vibrations throughout all experiments were recorded continuously in the vertical plane with a waterproof piezo-electric accelerometer (Bruel and Kjaer Type 4333, sensitivity 20.6 mV/g, Bruel and Kjaer Type charge amplifier 2635), and in all three planes using a tri-dimensional waterproof geophone (SM-7 370 ohm, IO, 28.8 V/m/s). There was no significant difference in ambient vibration levels across experimental sessions. All instruments were calibrated before use as described in previous work[5].

Thresholds were determined using the well-defined staircase method[21]. In short, the animal is presented initially with the greatest amplitude, with the amplitude being reduced or increased with every positive or negative response respectively. The threshold is calculated as the amplitude of the stimulus which the animals reacted to on 50% of the presentations, taken as an average of ten iterations. The presence of specific behavioral indicators was used in each case as a marker for stimulus perception. A camera above the tank enabled remote observations of the animals to allow the experimenter to monitor behavior without introducing additional disturbance. One animal was tested per day, with 10 – 20 minutes in between frequency presentations, and 2 – 5 minutes between amplitude presentations to allow recovery. Additional experiments within the tank exposed the animals to a short clip of vibration, with the time taken to recover (s) after a startle response measured.

2.2 Responses observed and relevance to anthropogenic operations

In both species, behavioral responses to vibration exposure were prominent throughout experiments without any signs of habituation within trials, although there was some indication of a reduction in sensitivity after long periods (weeks) in the laboratory. Mussels exhibited full or partial closure of the valves at onset of the stimuli which were distinct from natural rhythms of feeding. Hermit crabs showed a wider range of responses including the onset or cessation of movement, clear antennae and antennule movement for the duration of the signal. There were no responses to control exposures. In terms of velocity, sensitivity of animals ranged from 0.00001 – 0.006 m s^{-1} (crab, n = 45) and 0.00005 - 0.002 m s^{-1} (mussel, n = 15), 5 - 410 Hz (vertical plane), (full data *in press*). Greatest sensitivity for crabs was at 410 Hz compared to 10 Hz for mussels. Sensitivities fell within the ranges of sensitivities of other invertebrate species to particle motion, such as crustaceans and cephalopods[15,22-26], but there are no existing data regarding *M. edulis* and *P. bernhardus* to allow direct comparisons. *P. bernhardus* and *M. edulis* appear to be less sensitive to vibration than benthic fish[27,28], although it is difficult to make comparisons due to markedly different methodologies.

A comparison of threshold values to those measured in the vicinity of anthropogenic operations contacting the sea or riverbed indicated that such activities are likely to be detectable by the animals, and to have behavioral implications even at larger distances from the source. For example, vibrations produced by dredging and blasting at 220 - 300 m from operation fall within the behavioral threshold range. Other activities such as drilling, pile driving and shell auger are likely to elicit responses at 30 – 70 m distance (full data *in press*). It is important to note that only three specific behavioral indicators were tested here. The threshold of other behaviors (e.g. withdrawal of crab into shell) may differ, as may the thresholds for physiological changes or injury (which was beyond the scope of the current work).

In both species there was evidence to suggest that the duration of response varied depending on the strength of vibration exposure. In hermit crabs, duration in the laboratory prior to tests significantly affected results, with those fresh to the laboratory showing increased sensitivity compared to their more acclimated counterparts.

3 STUDYING SENSITIVITY TO VIBRATION

3.1 Universal methodologies

Work in small laboratory tanks, with numerous reflective boundaries, makes the acoustic field unpredictable[29-31]. Methods to overcome this difficulty may include standing wave tanks which aim to control both particle motion and pressure[11,14,25], or speakers positioned above the water surface [32]. In studies aiming to excite predominantly the substrate, the extent to which these factors have an influence upon the test animals are largely untested. However, in the case of many benthic invertebrates there is no evidence to support the detection of pressure[9,10,16,33] hence only water-borne stimuli may be appropriate. Since the boundary between water-borne and substrate-borne particle motion in liquids is unclear, this too may not be of relevance, especially since the same receptors may be used for detection of both. Nonetheless, it is a necessity to measure the vibratory field within the tank using calibrated sensors to measure all three planes of motion (i.e.- by using a tri-dimensional geophone, or three piezo-electric accelerometers). Measurements should be taken across the experimental tank to understand the variation in exposure by area. In the current work, fully calibrated sensors were used to measure the vibration upon the substrate.

The experimental tank itself must be built to minimize the influence of external vibrations upon the test subjects. This is important since there is a prevalence of background vibrations in the anthropogenic environment, so much so that investigations of terrestrial animals sensitivity to vibrations is said to be hindered by 'bioseismic' pollution[34]. Experimental tanks may be isolated from surroundings using layers of hard and soft material such as dampeners and gaskets [17,35], or by a suspended structure[36]. Such measures are necessary since there is evidence that sensory thresholds of fish, for example, are affected by background levels of vibration[37]. The effectiveness of such vibration reductions must be measured, and as with all vibroacoustic studies background levels must be measured throughout experiments to ensure that there are no additional stimuli within the experiments, as in the current work.

Shaker tables, constraining the bulk of the stimulus to one axis may be used to create a vibration stimuli[35]. Similar methods have been used in the exposure of semi-terrestrial crustaceans to vibration, by the use of a platform built upon a speaker cone[7,38]. Additionally stinger rods may be used to excite the substrate, as in the current work. In all cases, the stimulus must be measured in terms of frequency composition to ensure that the desired frequency is predominant as is necessary for auditory studies[39,40], and the proportion of energy within each plane of motion. In the current work, an electromagnetic shaker was used to excite the substrate of the tank via a stinger rod. The coupling between the stinger rod and the substrate was adjusted to minimize wave distortion, measurements were taken across the tank, and there were prominent energy peaks at the presented frequencies. However whilst the vertical plane was the strongest component of the signal, the stimulus was not constrained to one axis fully due to the nature of the setup.

Whilst restricting the mobility of the animal is preferable to ensure continuity of experimental signal between animals[7,38], such restriction may impede natural behavior. The experimental setup is then a compromise between stimulus purity and a setup allowing the observation of natural behaviors. This trade-off has been addressed in various ways, from full restriction such as slings, clamps and harnesses[6,7,41] to no restraint at all [42]. In all cases, the ideal scenario involves laser Doppler vibrometry to measure the received stimulus directly on the body of the test animals[25,43]. In the current work animals were unrestrained to allow full movement. Animals were also allowed acclimation time within the experimental setup, both these aspects were deemed crucial to obtaining accurate thresholds. In the current work, animals were observed for natural behaviors rather than being conditioned to respond to the stimulus. Behavioral conditioning, whilst common for fish audiometry studies[40] is more difficult for crustaceans due to, for example, naturally erratic heart rates[44]. The approach undertaken in the current work, using clear postural changes of the body has been successful in other studies[25,33,42,45,46].

3.2 Research directions

The link made here, between sensory thresholds and actual anthropogenic noise data, highlights that the sensitivity of these species is sufficient to allow the detection of seabed vibrations produced by anthropogenic activities within the marine environment. The behavioral indicators observed here may be short-lived, however the implications of these changes may be far reaching, affecting energy budgets, and 'distracting' animals away from foraging, reproductive and anti-predator behavior as seen in acoustic studies[47-51]. In the case of mussels for example, disruption of natural periodic valve movements is likely to affect the energy budget of the animals, which may then have implications for the productivity of mussel beds; of commercial and ecosystem consequences. The energy budget and body conditions of mussels may be easily monitored within laboratory conditions, but was beyond the scope of the current study[52-55].

Now that a sensory threshold is documented for these species, further work could explore the extent to which behavioral, physical and physiological changes may be present after exposure to vibration, as well as repeating the method with different species. It would also be valuable to investigate the threshold for injury. Further investigations would allow the translation of individual-level effects up to the population scale, contributing to the limited understanding about the impacts of seabed vibration upon benthic organisms.

As common for sensory threshold studies, the current work used a sinusoidal signal. Future work could use a similar experimental set up to investigate responses to other signals, such as short pulses, more continuous signals and anthropogenic recordings. Work to this effect has indicated responses to playback of shell auger piling which are likely to affect energy budgets of the animal[5].

Of most importance is a repetition of the current work within the field environment. Laboratory conditions cannot replicate the conditions these species encounter upon the seashore, in terms of physical and biological conditions, nor do they aim to. For example it is likely that behavior within the laboratory is different than in more natural conditions[56], and background levels of vibration may affect thresholds as found in work with fish[37].

Finally, the comparison here of anthropogenic values and thresholds is somewhat crude due to the use of a relatively small set of measurements, hindered by a lack of publicly available data. Vibrations produced by marine constructions are likely to vary with, for example, seabed type, environmental conditions, type of construction method, equipment used and propagation conditions; However there are few data available for use.

4 CONCLUSIONS

Behavioral thresholds to sinusoidal substrate-borne vibration were found for *P. bernhardus* and *M. edulis.* The results are a valuable first step in understanding the sensitivity of these species to vibration, which may be produced naturally or by man-made activities. The short term behaviors documented here are part of a suite of behaviors exhibited upon vibration exposure, and the consequences of these in the longer term now need to be explored. As with all behavioral studies the results must be considered within the experimental context, including the stimulus type, exposure length and also biological conditions such as natural variation between individuals.

When related to measurements of vibration-generating activities undertaken in the marine environment, the thresholds indicate that such activities would be detectable and induce behavioral changes. The thresholds obtained also provide an understanding of the levels of natural seabed vibration that may be detected by these species.

The sensitivity of these common coastal species is of great relevance due to the increasing trend of marine developments around coastlines, and the growing concerns relating to their effect upon the environment. The current data are a first step towards understanding the impact of anthropogenic vibration upon these organisms. More tests are required to establish the sensitivity of these, and other species, within the natural environment.

5 ACKNOWLEDGMENTS

We are grateful to the UK Department for Environment, Food and Rural Affairs (DEFRA) for funding the doctorate that formed this work. Thank you also to A. Hawkins (Loughine Ltd.), and R. Pérez-Domínguez (APEM) for discussions and support throughout, S. Jennings and V. Swetez for animal husbandry, and Subacoustech Ltd., Alstom Energy, Van Oord UK and AECOM UK for anthropogenic vibration data.

6 REFERENCES

1 Hazelwood, R. A. & Macey, P. in *The Third International Conference on The Effects of Noise on Aquatic Life* Vol. 1 (eds A. D. Hawkins & A.N. Popper) (Springer, Budapest, 2015).

2 Markl, H. in *Neuroethology and Behavioral Physiology* (eds F. Huber & H. Markl) 332-353 (Springer-Verlag, Berlin., 1983).

3 Aicher, B. & Tautz, J. Vibrational communication in the fiddler crab *Uca pugilator*. *J. Comp. Physiol., A.* **166**, 345-353, doi:10.1007/bf00204807 (1990).

4 Frings, H. & Frings, M. in *Marine Bio-Acoustics* (ed W.N. Tavolga) 261-282 (Pergamon Press, 1967).

5 Roberts, L. *Behavioural responses by marine fishes and macroinvertebrates to underwater noise* Ph.D. thesis, University of Hull, (2015).

6 Horch, K. An organ for hearing and vibration sense in the ghost crab Ocypode. *J. Comp. Physiol., A.* **73**, 1-21, doi:10.1007/bf00297698 (1971).

7 Salmon, M. & Atsaides, S. P. Sensitivity to substrate vibration in the fiddler crab, *Uca pugilator*. *Anim. Behav.* **17, Part 1**, 68-76, doi:10.1016/0003-3472(69)90114-6 (1969).

8 Hawkins, A. D., Pembroke, A. E. & Popper, A. N. Information gaps in understanding the effects of noise on fishes and invertebrates. *Rev. Fish Biol. Fish.*, 1-26, doi:10.1007/s11160-014-9369-3 (2014).

9 Breithaupt, T. & Tautz, J. in *Frontiers in crustacean neurobiology* (eds K. Wiese *et al.*) Ch. 1, 561 (Springer-Verlag, 1990).

10 Goodall, C., Chapman, C. J. & Neil, D. in *Frontiers in crustacean neurobiology* (eds K. Wiese, W.-D.
Tautz Krenz, J., H. Reichert, & B. Mulloney) Ch. 1, 561 (Springer-Verlag, 1990).

11 Plummer, M. R., Tautz, J. & Wine, J. J. Frequency coding of waterborne vibrations by abdominal mechanosensory interneurons in the crayfish, *Procambarus clarkii*. *J. Comp. Physiol., A.* **158**, 751-764 (1986).

12 Breithaupt, T. & Tautz, J. Vibration sensitivity of the crayfish statocyst. *Naturwissenschaften* **75**, 310-312 (1988).

13 Monteclaro, H. M., Anraku, K. & Matsuoka, T. Response properties of crayfish antennules to hydrodynamic stimuli: functional differences in the lateral and medial flagella. *J. Exp. Biol.* **213**, 3683-3691 (2010).

14 Tautz, J. & Sandeman, D. C. The detection of waterborne vibration by sensory hairs on the chelae of the Crayfish *Orconectes limosus*. *J. Exp. Biol.* **88**, 351-356 (1980).

15 Breithaupt, T. T., J. in *Frontiers in Crustacean Neurobiology* (1990).

16 Popper, A. N., Salmon, M. & Horch, K. Acoustic detection and communication by decapod crustaceans. *J. Comp. Physiol., A.* **187**, 83-89 (2001).

17 Mosher, J. I. The responses of *Macoma balthica* (bivalvia) to vibrations. *Proceedings of the Malacological Society of London* **40** (1972).

18 Kastelein, R. A. Effects of vibrations on the behaviour of cockles (bivalve molluscs). *Bioacoustics* **17**, 74-75 (2008).

19 Ellers, O. Discrimination among wave-generated sounds by a swash-riding clam. **189**, 128-137 (1995).

20 Roberts, L. & Breithaupt, T. in *The Third International Conference on The Effects of Noise on Aquatic Life* (Springer *In Press 2015*, Budapest, 2015).

21 Cornsweet, T. N. The staircase-method in psychophysics. *The American Journal of Psychology* **75**, 485-491, doi:10.2307/1419876 (1962).

22 Salmon, M. & Horch, K. Vibration reception in the fiddler crab, *Uca minax Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **44**, 527-541 (1973).

23 Aicher, B. & Tautz, J. 'Peripheral inhibition' of vibration-sensitive units in the leg of the fiddler crab *Uca pugilator*. *J. Comp. Physiol., A.* **154**, 49-52, doi:10.1007/bf00605389 (1984).

24 Hughes, A. R., Mann, D. A. & Kimbro, D. L. Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* **281**, doi:10.1098/rspb.2014.0715 (2014).

25 Breithaupt, T. in *The crustacean nervous system* (ed K. Wiese) 548-449 (Springer, 2002).

26 Kaifu, K., Akamatsu, T. & Segawa, S. Underwater sound detection by cephalopod statocyst. *Fish. Sci.* **74**, 781-786, doi:10.1111/j.1444-2906.2008.01589.x (2008).

27 Chapman, C. J. & Sand, O. Field studies of hearing in two species of flatfish *Pleuronectes platessa* (L.) and *Limanda limanda* (L.) (family Pleuronectidae). *Comp. Biochem. Physiol., A: Mol. Integr. Physiol.* **47A**, 371-385. (1974).

28 Sigray, P. & Andersson, M. H. Particle motion measured at an operational wind turbine in relation to hearing sensitivity in fish. *The Journal of the Acoustical Society of America* **130**, 200-207 (2011).

29 Parvulescu, A. in *Marine Bio-Acoustics* (ed W. N . Tavolga) 87-100 (Pergamon Press, 1964).

30 Parvulescu, A. in *Marine Bio-Acoustics* Vol. 2 (ed W.N. Tavolga) 7-13 (Pergamon Press, 1964).

31 Rogers, P. in *The Third International Conference on The Effects of Noise on Aquatic Life* (eds A. Hawkins & A.N. Popper) (Springer *In Press 2015*, Budapest, 2015).

32 Neo, Y. Y. *et al.* Behavioral changes in response to sound exposure and no spatial avoidance of noisy conditions in captive zebrafish. *Frontiers in Behavioral Neuroscience* **9**, 28, doi:10.3389/fnbeh.2015.00028 (2015).

33 Goodall, C. *The sensory detection of water borne vibrational stimuli and their motor effects in the norway lobster, Nephrops norvegicus (L.)* Doctor of Philosophy thesis, Glasgow University, (1988).

34 O'Connell-Rodwell, C. E., Hart, L. A. & Arnason, B. T. Exploring the potential use of seismic waves as a communication channel by elephants and other large mammals. *Am. Zool.* **41**, 1157-1170, doi:10.1093/icb/41.5.1157 (2001).

35 Mooney, T. A. *et al.* Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. *J. Exp. Biol.* **213**, 3748-3759, doi:10.1242/jeb.048348 (2010).

36 Karlsen, H. E. Infrasound sensitivity in the plaice (*Pleuronectes Platessa*). *J. Exp. Biol.* **171**, 173-187 (1992).

37 Hawkins, A. D. & Chapman, C. J. Masked auditory thresholds in the cod, *Gadus morhua* L. *J. Comp. Physiol., A.* **103**, 209-226, doi:10.1007/bf00617122 (1975).

38 Salmon, M. Signal characteristics and acoustic detection by the fiddler crabs, *Uca rapax* and *Uca pugilator*. *Physiol. Zool.* **44**, 210-224, doi:10.2307/30152493 (1971).

39 Chapman, C. J. & Hawkins, A. D. A field study of hearing in the cod, *Gadus morhua* (L.). *J. Comp. Physiol., A.* **85**, 147-167, doi:10.1007/bf00696473 (1973).

40 Ladich, F. & Fay, R. Auditory evoked potential audiometry in fish. *Rev. Fish Biol. Fish.* **23**, 317-364, doi:10.1007/s11160-012-9297-z (2013).

41 Horch, K. The acoustic behavior of the Ghost Crab *Ocypode cordimana* Latreille, 1818 (Decapoda, Brachyura). *Crustaceana* **29**, 193-205 (1975).

42 Heinisch, P. & Wiese, K. Sensitivity to movement and vibration of water in the North Sea shrimp *Crangon crangon* L. *J. Crust. Biol.* **7**, 401-413 (1987).

43 Aicher, B., Markl, H., Masters, W. M. & Kirschenlohr, H. L. Vibration transmission through the walking legs of the fiddler crab, *Uca pugilator*, (Brachyura, Ocypodidae) as measured by laser doppler vibrometry. *J. Comp. Physiol., A.* **150**, 483-491, doi:10.1007/bf00609574 (1983).

44 Florey, E. & Kriebelm, M. E. The effects of temperature, anoxia and sensory stimulation on the heart rate of unrestrained crabs. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **48A**, 285-300 (1974).

45 Berghahn, R., Wiese, K. & Ludemann, K. Physical and physiological aspects of gear efficiency in North Sea brown shrimp fisheries. *Helgoländer Meeresuntersuchungen* **49**, 507-518 (1995).

46 Tautz, J. Water vibration elicits active antennal movements in the crayfish, *Orconectes limosus*. *Anim. Behav.* **35**, 748-754, doi:10.1016/s0003-3472(87)80111-2 (1987).

47 Hawkins, A. D., Roberts, L. & Cheesman, S. Responses of free-living coastal pelagic fish to impulsive sounds. *The Journal of the Acoustical Society of America* **135**, 3101-3116, doi:doi:http://dx.doi.org/10.1121/1.4870697 (2014).

48 Simpson, S. D., Purser, J. & Radford, A. N. Anthropogenic noise compromises antipredator behaviour in European eels. *Global Change Biol.* **20**, doi:10.1111/gcb.12685 (2014).

49 Chan, A.-H., Perez, P., Smith, S. & Blumstein, D. J. Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. *Biol. Lett.* **6**, 458-461 (2010).

50 Chan, A. A. Y.-H. *et al.* Increased amplitude and duration of acoustic stimuli enhance distraction. *Anim. Behav.* **80**, 1075-1079, doi:http://dx.doi.org/10.1016/j.anbehav.2010.09.025 (2010).

51 Wardle, C. S. *et al.* Effects of seismic air guns on marine fish. *Cont. Shelf Res.* **21**, 1005-1027, doi:Doi: 10.1016/s0278-4343(00)00122-9 (2001).

52 Mazik, K. *et al.* Sublethal effects of a chlorinated and heated effluent on the physiology of the mussel, *Mytilus edulis* L.: a reduction in fitness for survival? *Mar. Pollut. Bull.* **77**, 123-131, doi:http://dx.doi.org/10.1016/j.marpolbul.2013.10.020 (2013).

53 Widdows, J. *et al.* Measurement of stress effects (scope for growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. *Mar. Environ. Res.* **53**, 327-356, doi:http://dx.doi.org/10.1016/S0141-1136(01)00120-9 (2002).

54 Widdows, J., Nasci, C. & Fossato, V. U. Effects of pollution on the scope for growth of mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy. *Mar. Environ. Res.* **43**, 69-79, doi:http://dx.doi.org/10.1016/0141-1136(96)00003-7 (1997).

55 Widdows, J. *et al.* Relative importance of environmental factors in determining physiological differences between two populations of mussels *(Mytilus edulis)*. *Mar. Ecol. Prog. Ser.* **17**, 33-47 (1984).

56 Benhaïma, D. *et al.* Early life behavioural differences in wild caught and domesticated sea bass (*Dicentrarchus labrax*). *Appl. Anim. Behav. Sci.* **141**, 79-90 (2012).