

Behavioural effects of hypersaline exposure on the lobster *Homarus gammarus* (L) and the crab *Cancer pagurus* (L)

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Abstract

There is scarce existing information in the literature regarding the responses of any marine species, especially commercially valuable decapod crustaceans, to hypersalinity. Hypersaline discharges due to solute mining and desalination are increasing in temperate areas, hence the behavioural responses of the edible brown crab, *Cancer pagurus*, and the European lobster, *Homarus gammarus*, were studied in relation to a marine discharge of highly saline brine using a series of preference tests. Both species had a significant behavioural response to highly saline brine, being able to detect and avoid areas of hypersalinity once their particular threshold salinity was reached (salinity 50 for *C. pagurus* and salinity 45 for *H. gammarus*). The presence of shelters had no effect on this response and both species avoided hypersaline areas, even when shelters were provided there. If the salinity of commercial effluent into the marine environment exceeds the behavioural thresholds found here, it is likely that adults of these species will relocate to areas of more favourable salinity. In management terms it is advisable to ensure that any hypersaline discharges are limited to the lowest tolerance of all the economically valuable species in the area to avoid loss of revenue in fishery areas.

1. Introduction

Cancer pagurus, the edible brown crab (Linnaeus 1758) and *Homarus gammarus*, the European lobster (Linnaeus 1758) are both stenohaline, osmoconforming species that are generally subtidal but can occur in the lower intertidal zone. Most existing literature on salinity change in *H. gammarus* relates to their physiological responses to hyposaline conditions (Charmantier et al., 1984; Lucu and Devesconi, 1999; Pavičić-Hamer et al., 2003) and for *C. pagurus* there are no such studies. Likewise, little is known of either species' response to hypersaline challenge. In their natural environments, temperate crustaceans that are generally fully marine in nature rarely, if ever, experience hypersalinity hence the lack of attention to this subject. The principal focus of studies that have been made on high salinities relates to the effect of desalination plant discharges in hot climates (Meerganz von Medeazza, 2005; Raventos et al., 2006; Smith et al., 2007) or species that live in saltpan and saline lakes that have high evaporation rates (Clegg and Gajardo, 2009; Nunes et al., 2006) or mangrove swamps (Anger and Charmantier, 2000; Gillikin et al., 2004). Because hypersaline conditions are relatively scarce in temperate regions there is correspondingly less information on the effects of hypersalinity on temperate species. A consequence of increasing worldwide demands for fresh water is an increased interest in desalination in all regions — including temperate areas. Currently, desalination plants are principally located in the southern areas of the Northern Hemisphere (e.g. the Middle East and the Americas), where low rainfall limits the availability of fresh water (Raventos et al., 2006). Desalination, however, is now increasing in more northerly areas such as the European side of the Mediterranean Sea, and has been taking place on the islands of Jersey and Guernsey since the 1970s (Romeril, 1977) and in mainland UK since 2010 (Li et al., 2011). An additional, recent source of hypersalinity in the marine environment is solute mining when creating underground caverns for the storage of natural gas and for carbon sequestration (Bérest et al., 2001; Dusseault et al., 2001; Quintino et al., 2008).

Industries such as these have inevitably been accompanied by the need to discharge the resultant brine at sea with the concomitant impact this may have on the marine fauna local to the point of discharge. Such discharge activities occur offshore from the coast of Portugal (Quintino et al., 2008) and the UK (Evans, 2008) for example. The brine is discharged through a diffuser to disperse it rapidly and thereby reduce its environmental impact but, within the discharge plume, ambient salinity is increased (Cutts et al., 2004), with discharges from

desalination being up to approximately 2.5 times the salinity of full seawater (salinity of ≈ 90) (Fernández-Torquemada et al., 2005) and solute mining up to 8.5 times (salinity of ≈ 300) (Quintino et al., 2008). Commonly, discharges are made in coastal waters which often support commercial shellfisheries. For example a solute mining discharge off the coast of East Yorkshire, UK, is sited within an area that supports nationally and locally important fisheries for *H. gammarus* and *C. pagurus*, which contribute significantly to the economy (Walmsley and Pawson, 2007).

Environmental salinity influences the reproduction, larval dispersal, larval recruitment and geographical distribution of marine crustaceans (Anger, 1991, 1996; Spivak and Cuesta, 2009) and therefore has the potential to influence growth, mortality, health and immune functions and reproductive success, hence salinity changes are likely to impact on crustacean population dynamics. Consequently, the ecological effects of increased hypersaline discharges may also have a significant and widespread commercial relevance. This is true in terms of the success of fishing and post-harvest marketing operations as well as having potentially negative impacts on larval recruitment and stock replenishment.

Altered overt behaviour (behaviours such as limb, mouthpart or body movements, rather than concealed behaviour such as heart or scaphognathite beat changes) is usually the first response to changed salinity and this can help organisms avoid adverse conditions (Curtis et al., 2007). One survival strategy employed by aquatic crustaceans when challenged with high stressor intensities in their environments (such as salinity changes, predators, vibration, noise), is an escape or avoidance response, (e.g. a movement of the whole or part of the organism) away from the affected area (e.g. by fleeing, retreating into a burrow or protective shell, or as with barnacles, withdrawing behind protective opercular plates) (Kinne, 1964). The failure of a behavioural response system can lead to reduced individual fitness and associated adverse consequences for the population (Miller, 1980). Habitat structure can influence the physiological and behavioural mechanisms of organisms directly and must be considered when interpreting the responses of animals in relation to physicochemical variables (McGaw, 2001). For many lobster and crab species, the presence of shelter may induce a crustacean to stay in an area of high salinity when conditions become sufficiently unfavourable as to otherwise cause it to vacate the area (Cobb, 1971b; Howard and Nunny, 1983; McGaw, 2001; Shumway, 1978; Smith and Herrkind, 1992; Spanier and Almog-Shtayer, 1992; Spanier and Zimmer-Faust, 1988). Physical factors and seabed topography have been shown to affect the size composition of *H. gammarus* and *Homarus americanus* populations, with substratum type and current strength also having major influences (Howard and Nunny, 1983; Robichaud and Campbell, 1991).

It is hypothesised here that there are quantifiable changes to the behaviour of *H. gammarus* and *C. pagurus* in response to hypersaline media. This hypothesis has been tested with a view to providing information to aid the understanding of the potential sustainability of commercially important crustacean species facing such stresses. Such studies are important, given the imminence of increased gas cavern, carbon sequestration and desalination plant construction projects worldwide and their potential impacts on international commercial crustacean fisheries.

The aim of this study was to determine whether and, if so, to what extent, hypersalinity causes halokinesis/halotaxis (a movement in response to salinity) in *H. gammarus* and *C. pagurus*, and, whether the presence of a shelter would affect the salinity preferences of these species. For the purposes of this investigation, hypersalinity is defined as any salinity above, and hyposalinity any salinity below, that which the species experience normally in the wild in an open temperate marine area. In the case of the animals tested here, normal salinity is 35. The practical salinity scale, which has no units, is used here to state salinity (UNESCO, 1985).

2. Methods

Creel-caught, intermoult specimens of minimum landing size and up to 4 mm above *C. pagurus* (130 mm carapace width) and *H. gammarus* (87 mm carapace length), were obtained from local commercial landings at Bridlington, East Yorkshire, UK, where the environmental salinity is 35. Animals were transported, dry, to the laboratory (ca 1 h journey) where they were kept in a temperature controlled room ($8^{\circ}\text{C} \pm 1^{\circ}\text{C}$),

unfed, in aerated seawater ($8\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, salinity of 35 ± 1) for 5 days before being used in experiments to allow recovery from capture, transport and handling. Both the crabs and lobsters were maintained prior to experimentation with their chelae bound, in opaque plastic tanks at a stocking density of 2 animals per tank and an average of 3 L of water per individual. A 12 h artificial dim light/dark cycle was maintained throughout holding and experimentation. The holding water was changed daily (50%) via an unobtrusive drain/refill method. All experiments were performed in a screened off area of the temperature controlled room, thus maintaining a consistent air and water temperature.

Hypersaline brine was produced using natural seawater from Bridlington, UK (salinity 35) as a base then adding Instant Ocean aquarium salts to raise the salinity. Instant Ocean is a clean and sterile product and was used so that a salinity-only response could be studied, rather than one prompted by any other chemicals that may be present if the water had been sourced from existing brine discharges. Results are thus pertinent to a variety of areas affected by hypersaline conditions. All seawater and brine solutions were allowed to settle at the room temperature before used.

2.1. Two choice trial

A two-way choice chamber was constructed (Fig. 1) consisting of two roughened plastic slopes to allow the crustaceans purchase, with a flat central apex inside a large opaque plastic tank, (100 cm * 35 cm * 25 cm) to allow separate pools of water on each side of the apex. Different choices of salinity were given with one side always being at the normal salinity of 35, then the other side being a higher test salinity within the range of 35 to 65 (in increments of 5), giving a total of 7 different salinity pairings. No mixing occurred between the two pools which each maintained a stable salinity. A test animal could still crawl easily over the central apex. Pools were aerated gently and maintained at a constant temperature ($8\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$) to minimise the number of variables that could affect the choice response of test specimens. A parallel control experiment using identical numbers of new animals was also conducted using with both pools at salinity 35 to ensure there was no preference for a certain side and to ensure there were no other factors controlling any salinity preferences. Tests were run only during the 12 h light period (dim light).

C. pagurus ($n = 30$) and *H. gammarus* ($n = 20$) were used for each choice duo (total 210 *C. pagurus*, 120 *H. gammarus*). Animals were used only once to avoid any effects of acclimation. Each test involved the placement of a single specimen in the chamber at the apex of the slopes thus presenting it with the choice of remaining emersed (out of water) or descending into either pool of water. The animal was observed from behind a screen and the time taken to move into a pool and the pool choice made were recorded. Upon placement into the experiment, both the crabs and lobsters proceeded to dip their legs into both salinity options, before making a choice. On the assumption that a 6 h emersion period represented the maximum time for which a mid to low shore animal would be tidally emersed in a typical 12.2 h tidal cycle, those animals that took longer than 6 h to make a choice were deemed as being unresponsive, however the majority of animals made their choice within the first few minutes and none reached the 6 h cut-off mark. A positive choice was counted when the animal had its legs and mouthparts submerged and was therefore able to ventilate its gill chambers thus wetting the principal sites of salinity detection in these species (Dr. R. Uglow *pers. comm.*, Dufort et al., 2001) although all animals did fully enter the pools of water. The experiment was considered finished once the choice of pool had been made. If, after choosing, the animal changed pools before it could be removed from the test chamber, only the initial choice was recorded. This decision was made because, for the purposes of this experiment, only the initial response to salinity was being considered, rather than one which may have occurred after a period of acclimation in the chosen pool.

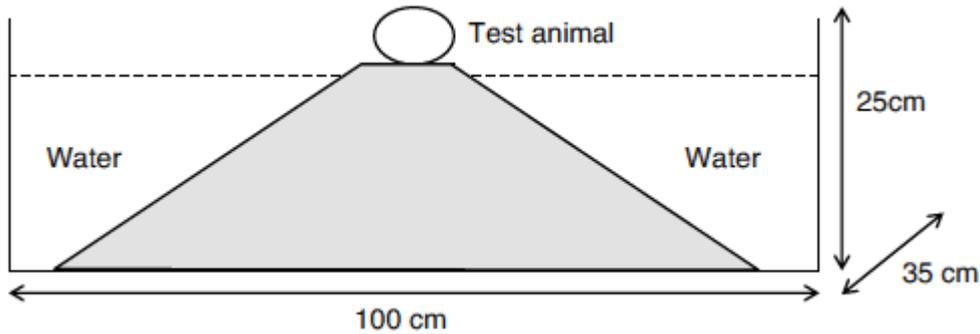


Fig. 1. Cross sectional schematic representation of the two-choice testing chamber (100 cm * 35 cm * 25 cm). Not to scale. Size of *Homarus gammarus* used: 8.7 cm–9.1 cm carapace length. Size of *Cancer pagurus* used 13.0 cm–13.4 cm carapace width.

2.2. Multi-choice trial

The aim of these tests was to determine whether the test animals would prefer a sheltered location with hypersaline conditions, or an exposed location of normal (35) salinity. This was done in order to help describe what might happen should an area of seabed inhabited by these species come under the influence of a hypersaline discharge. The experimental set-up comprised a circular tank (depth 60 cm, diameter 80 cm), filled with sand to a depth of 15 cm into which 4 shelters were buried at a 30° angle (Fig. 2). Each shelter was constructed from a 2 L soft plastic bottle with the rounded neck removed to create a large opening. Each was strengthened by sections of round pipe and each had a seawater inlet fixed at the rear. Shelters were connected via the delivery inlet to a peristaltic pump, with 3 hypersaline reservoirs and one isosaline reservoir (both cf. normal seawater salinity of 35) (Fig. 2). The pumps were calibrated so that each delivered a slow trickle flow of 2 ml·min⁻¹ to the shelters. The salinity of each reservoir was calibrated so that, on mixing with the normal salinity of 35 seawater in the experimental tank, the salinity in the shelters would equate to salinities 80–90, 60–70, 40–50, or 35–40. As the density of hypersaline water is greater than that of normal seawater, the low flow rate into the shelters ensured that the hypersaline test conditions were confined to the shelter only. When the water in each shelter had reached the desired salinity, a single test animal was introduced to the centre of the tank and the set-up left for 24 h. *C. pagurus* (n = 16) and *H. gammarus* (n = 20) were used. Tests were performed individually over successive days. Animals were used only once to avoid any effects of acclimation and could explore the tank and shelters freely throughout the duration of the experiment, allowing a choice to be made as to where in the tank to settle. On completion of the test (24 h) the position of the specimen was recorded and taken as the choice made. The salinity of the shelters and open areas of the tank were checked every 15 min during the first 4 hand then hourly for 4 hand a final check after 24 h, using 0.5 ml water samples collected via a syringe that was able to reach the bottom of the tank. Salinity was determined using a calibrated refractometer.

A parallel control test was run, using identical numbers of new animals (16 *C. pagurus* and 20 *H. gammarus*), in which the shelters all received a flow of salinity 35 seawater to ensure there was no preference for a particular shelter or that the flow was not affecting the behaviour. These shelters were numbered from 1 to 4 with 1 being in the same position as the 80–90 shelter in the hypersaline trial, 2 being located as per the 60–70, 3 as per the 40–50, and 4 as per the 35–40.

2.3. Statistical analyses

Behavioural choices were analysed using a G-test (Fowler et al., 1998), with a Ln (x + 1) transformation due to trials in which zero observed values occurred for some choice options (Dr Jim Fowler, *pers. comm*). The G-test was used because of being unable to construct the 2 by 2 contingency table and hence the minimum 1 degree of freedom required for the normal χ^2 test. Expected values were calculated by apportioning the total number of observations to the available frequencies.

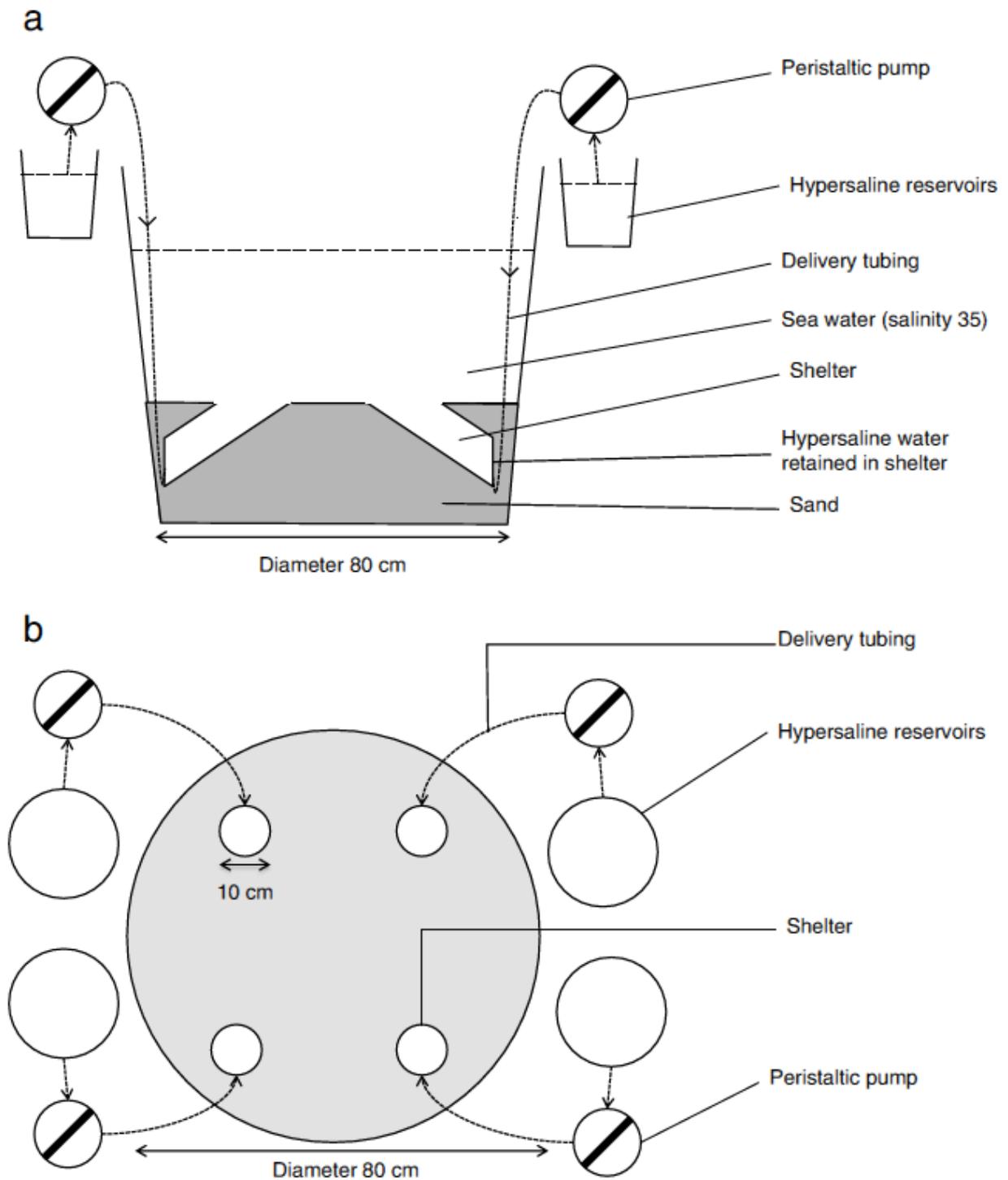


Fig. 2. (a) Cross sectional schematic representation of the multi-choice testing chamber (depth 60 cm, diameter 80 cm). Not to scale. Size of *Homarus gammarus* used: 8.7 cm–9.1 cm carapace length. Size of *Cancer pagurus* used 13.0 cm–13.4 cm carapace width. (b) top-down view of the multi-choice testing chamber (depth 60 cm, diameter 80 cm). Not to scale. Size of *H. gammarus* used: 8.7 cm–9.1 cm carapace length. Size of *C. pagurus* used 13.0 cm–13.4 cm carapace width.

3. Results

3.1. Two choice trial

In general, *C. pagurus* showed a preference for the normal salinity of 35. This preference increased from a 63.3% preference when the choice was salinity 35–40 to a 90% preference when the choice was salinity 35–65. Strong preferences for salinity 35 occurred from test salinity choices of ≥ 45 with over 75% choosing salinity 35 over the hypersaline option (Fig. 3). Although there was no significant preference for normal salinity over salinity 40 in the 30–40 choice test, *C. pagurus* did show a significant ($p < 0.01$) preference for the normal salinity (35) over a higher salinity once the choice became one between 35 and >40 (Fig. 3).

H. gammarus showed a preference for the normal salinity of 35 as the hypersaline option increased from ambient. The strongest preference occurred when the choice was 35–60 with 90% choosing salinity 35. Strong preferences for salinity 35 occurred from salinity >50 with over 75% of choosing salinity 35 over the hypersaline option (Fig. 4). Once the salinity choice offered with normal salinity (35) reached ≥ 50 in the hypersaline range, *H. gammarus* showed a significant ($p = 0.01$) preference for the ambient salinity (Fig. 4). At intermediate salinity choices there were no significant behavioural preferences shown.

There were no significant preferences shown in any of the control trials for either side of the tank ($p > 0.05$ in all cases).

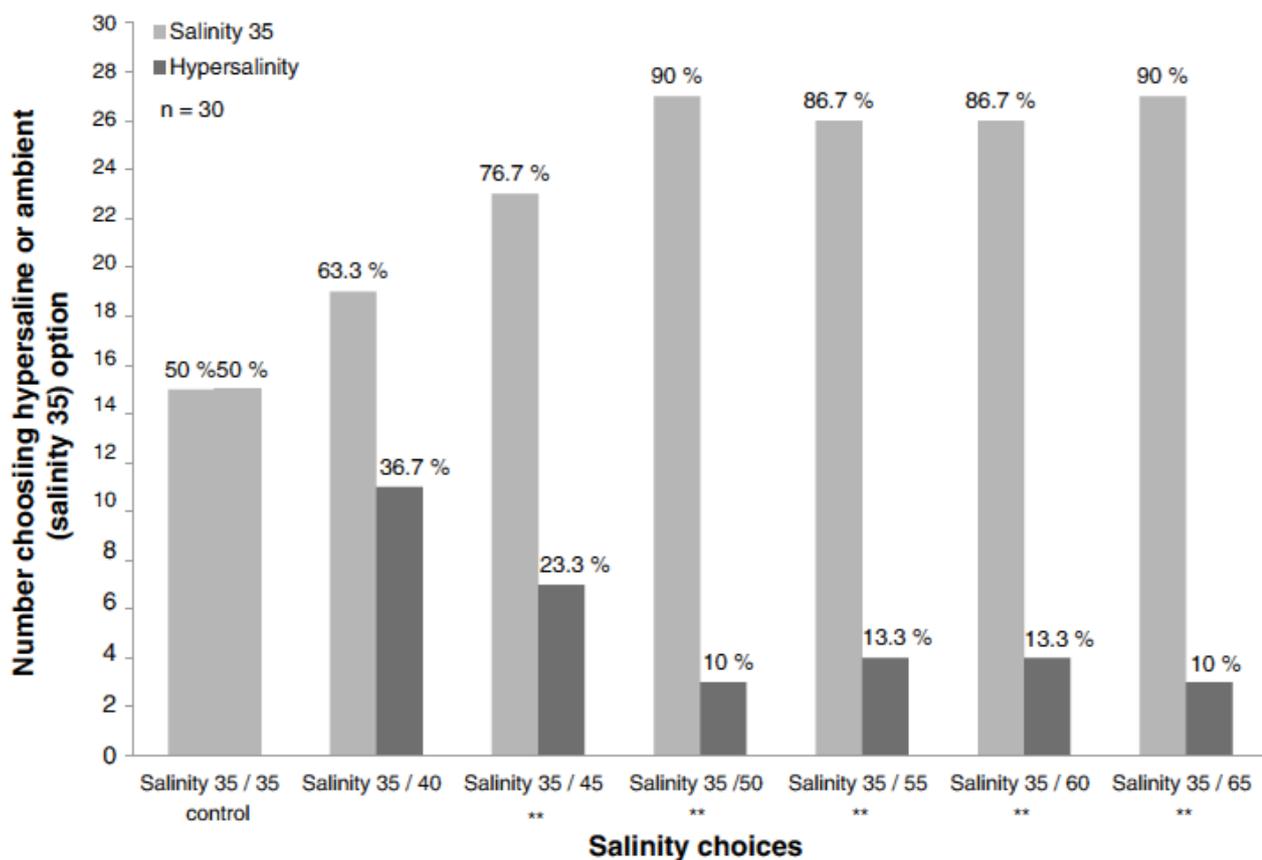


Fig. 4. Salinity preferences of *Homarus gammarus* when given the choice of salinity 35 plus a hypersaline option. ** denotes that there was a significant ($p < 0.01$) preference for salinity 35 in the G test (G values are: 35/50 = 10.55, 35/55 = 10.53, 35/60 = 14.63).

3.2. Multi choice trial

All but 4 of the *C. pagurus* chose the 35–40 salinity shelter or open water at salinity 35. Eight crabs chose the open water, four crabs chose the shelter at salinity 35–40, three crabs chose the shelter at 40–50 and one crab chose the shelter 60–70. In total, 75% of the test crabs avoided the hypersaline option offered which suggests strongly that they will show avoidance behaviour when exposed to a hypersaline environment.

When shelter or open water options are considered as separate choices, no significant preferences were shown by *C. pagurus* ($G = 4.407$, $df = 4$, $p > 0.05$) but when the open water choices are grouped together with the 35–40 psu shelter, if the latter was taken to be a “normal salinity” category, it is evident that there is a significant preference ($G = 8.710$, $df = 3$, $p < 0.05$) for normal salinities over hypersalinities.

All but one *H. gammarus* (19 out of 20) avoided the hypersaline areas of the tank in the multi-choice trial. Ten lobsters chose open water at salinity 35, nine chose the lowest salinity shelter (salinity 35–40). The only exception was a single lobster that chose the shelter at salinity 40–50. Thus lobsters, even when given the choice of a shelter, appear to avoid hypersalinity >40 .

There were statistically significant preferences for the shelter at the lowest hypersalinity of 35–40 and open water of salinity 35 ($G = 10.15$, $df = 4$, $p < 0.05$). A further G-test, combining the results for open water and the 35–40 shelter as a “normal salinity” category as for *C. pagurus*, also revealed a highly significant preference ($G = 18.88$, $df = 3$, $p < 0.01$) for normal salinity when exposed to a choice of normal salinity or various hypersaline options.

In the control trials, whereby all shelters received a flow of salinity 35 seawater, in the case of *C. pagurus* three crabs chose shelter 1, four chose shelter 2, four chose shelter 3, three chose shelter 4 and two chose to be in the open water. For *H. gammarus*, six lobsters chose shelter 1, three chose shelter 2, five chose shelter 3, two chose shelter 4 and four chose the open water. When tested as for the experimental trial, both when looking at the four shelters and the open water as 5 individual choices, and when considering shelter 4 and the open water as one category (so as to be consistent with the experimental trials), there were no significant preferences shown in the any of the control trials for either species ($p > 0.05$ in all cases).

4. Discussion

C. pagurus and *H. gammarus* are widely considered to be predominantly subtidal species, with adults only occasionally occurring in the lower littoral zone, probably when stranded in pools by the receding tide or as a consequence of onshore tidal scour for example during storm events (pers obs.). Subtidal species generally are considered as being iono- and osmoconformers (Péqueux, 1995), possibly because they are unlikely to become challenged by salinities other than that of normal seawater and their lack of much need for regulatory mechanisms. On that assumption, the threshold values for avoidance behaviour found here for these two species are higher than may have been expected. Here, in the two-choice trial, *C. pagurus* chose equally to be in normal seawater or in a hypersaline medium up to, but not exceeding salinity 50, a salinity of 15 above normal seawater. *H. gammarus* also showed no preference between normal and hypersaline media up to, but not exceeding, its avoidance behavioural threshold of salinity 45. These behavioural thresholds are approximately a salinity of 5 lower than the salinities that produce a 50% mortality in these species, where salinities over 48.9 and 55.5 are known to cause a 50% mortality in *H. gammarus* and *C. pagurus* respectively (Smyth, 2011). As no preference was shown in the control tests, these findings here and the findings of Smyth (2011) on physiological responses in these species, suggest that both species may be able to ion- and osmoregulate efficiently in a hypersaline medium up to their threshold value, even though it is presumed that naturally-occurring hypersaline challenges are rare in their natural environments. The fact that they exhibit a salinity choice suggests that salinities up to that level fall within the species' physiological tolerance zone, at least for a certain amount of time. This tolerance may then lead to acclimation which for the

purposes of this discussion is defined as the point at which an organism is considered to have adapted physiologically to survive normally in the new environment. A median lethal time of >500 h in a challenging environment is taken as indicative of survival (Davenport, 1972).

The literature reveals few investigations of the salinity-based responses of the two test species, and the lower and upper salinity preferences and tolerances of *C. pagurus* and *H. gammarus* are previously unknown. Such information is also scant for closely related species and what does exist pertains to the hyposaline range. However, *H. gammarus* has been acclimated successfully down to salinity 20 in the laboratory (Lucu and Devesconi, 1999) and the mortality of juveniles reared at 15 °C occurs only in salinities <17 and >46, with regulation being isosmotic in high salinities, and slightly hyperosmotic in low salinities (Charmantier et al., 1984).

The avoidance of hypersaline media shown by *H. gammarus* in the two-choice salinity tests described here, is not considered to be an experimental artefact. Although in relation to hyposaline rather than hypersaline conditions, *H. americanus* shows similar avoidance behaviour of salinities deviating from the norm, with salinity-dependent movements to deeper water in response to lowered salinity in its estuarine environments (Jury et al., 1994a, 1994b). This latter species has also been shown to differentiate between ambient and hyposaline media with a lower salinity limit of 12.6 that prompts such avoidance behaviour (Jury et al., 1994b).

C. pagurus is considered to be an osmoconformer (Péqueux, 1995) and the related species *Cancer borealis* and *Cancer irroratus* are also sub-littoral, osmoconforming crabs (Charmantier and Charmantier-Daures, 1991). The latter two species prefer rocky or gravelly bottoms, but can be found also on muddy/silty substrata (Robichaud and Frail, 2006). The 48 h lethal salinity (LS50) for adult *C. irroratus* occurs outside of the range of salinity 8.5–65. In *C. borealis*, the corresponding LS50 values are salinity >65 and <12. The adults of both species were isosmotic in high salinities and weak hyper-regulators in low salinities (Charmantier and Charmantier-Daures, 1991). The Dungeness crab, *Cancer magister* can detect changes in salinity at 29.9 and 32.7, values which correspond to 96% and 105% of its mean ambient salinity (salinity 31) (Sugarman et al., 1983). It is unknown whether these crabs also show preference behaviour associated with these LS50 values but it is known that, when exposed to a hyposaline gradient, the related osmoconforming *Cancer gracilis*, moves towards the higher salinities (Curtis et al., 2007), away from the unfavourable salinity. All species tested here in the two choice tests were able to discriminate between salinities and all made a choice of salinity 35 once their particular high salinity threshold was reached (50 for *C. pagurus*, 45 for *H. gammarus*).

In the multi-choice tests, both species showed a significant likelihood of choosing either a shelter with normal to low hypersaline conditions (35–40) or open water (where potentially exposed to predators), at salinity 35. No preference was exhibited in the control tests for any particular area of the tank. These findings indicate a preference for normal salinity over hypersaline conditions. The finding that lobsters chose equally to be either in a shelter at salinity 35–40 or in open water at 35, suggests that they would actively avoid hypersalinity in their environment and this raises a question whether such behaviour would have an influence on distributions of lobsters or crabs within areas affected by a brine plume. If an avoidance behaviour occurred, this could have implications for fisheries located in brine discharge areas if adult (i.e. commercially valuable) specimens relocate to more favourable habitats. The extent of area affected would depend on the nature of the discharge, the prevailing local conditions and size of the area over which the plume travelled.

The two species studied here are known for their use of shelters for many reasons including during times of moult or reproductive behaviour (Woll, 2003), in relation to light/dark cycles and as ways of ambushing prey (Ball et al., 2001; Lawton, 1989; Richards and Wickins, 1979; Smith et al., 1999) and lack of shelter is thought to be a limiting factor in the distribution of *H. gammarus* (Cobb, 1971a). In the multi-choice test, such use of shelter was shown to be 'over-ridden' by hypersalinity, with both species choosing either a shelter with a salinity of 35–40 or open water of salinity 35 in preference to shelters with more hypersaline media. Contrastingly, in the control tests, all locations in the tank were chosen equally. These findings suggest that, in the field, populations of these crustaceans will avoid areas of the seabed affected by a brine plume deriving from any solute mining or desalination process, regardless of whether there are opportunities for shelter in that area. Jury et al (1994b) also suggest that *H. americanus* uses behavioural adaptation to avoid potentially lethal salinities and although their research pertains to hypo- rather

than hypersalinity, it does give further evidence that Homarid lobsters make behavioural movements to avoid areas of unfavourable salinity, especially considering that salinities over 48.9 and 55.5 are known to cause a 50% mortality in *H. gammarus* and *C. pagurus* respectively (Smyth, 2011). The converse of this behaviour is true for the Pacific *Hemigrapsus nudus* which, when shelter is available, will endure longer exposures to salinities below its normal preference than it does when shelter is not available (McGaw, 2001). In the case of *H. nudus*, the benefits of shelter appear to outweigh the energetic costs of increased iono-osmoregulation when exposed to hyposaline conditions, however in a commercially fished species there may be implications of such behaviour on long term growth and survival.

Behavioural responses in the laboratory occur in a small space and over a short period of time, often, as is the case here, with abrupt changes in tested parameters such as salinity. Consequently, caution is always required when extrapolating such laboratory-based results to field conditions. For example, although a crab or lobster may seem, in a laboratory trial over ca. 24 h, to be comfortable in hypersaline conditions, if these conditions persisted in the long term the animal may choose to move back, if possible, to its normal salinity preference range. Salinity changes in the natural environment may also be gradual and will most likely occur over a large area which is subjected to natural currents and wave action. Dispersion and successive dilution of brine discharges are dependent on the prevailing hydrographic regime and mixing conditions. The strength of tidal, wind-driven and density driven currents will indicate the rate at which the brine disperses and mixes, hence giving the resultant dilution and salinity of the receiving waters. Under such conditions, it is possible that behavioural choices are less marked than those that furnished the results obtained here but, given sufficient time for a salinity change to develop, it is expected that behaviours as described will occur.

The data presented here are valuable given the previously limited knowledge of the behavioural responses to salinity change for the species studied and for related species. Hence it is of importance that there was a decreasing tolerance as salinities depart from normal and a significant movement away once a threshold hypersaline point is reached. This appears to be consistent with the related species for which some information is available. Furthermore, the use of a shelter which is common to the studied species does not override the adverse properties of high salinities.

5. Conclusion

The species tested are able to detect and discriminate between changes in salinity of magnitudes that are comparable to those that may be found in areas subjected to brine discharges. The responses can be summarised as an active avoidance of supra-threshold salinities, even when suitable shelter was available. Under the conditions tested, *C. pagurus* will choose ambient salinity (35) over areas where salinity is >40, and *H. gammarus* chooses ambient over areas of salinity ≥ 50 . Where multiple hypersalinity options are given, both species prefer areas of ambient salinity.

This increased knowledge of the tolerance thresholds when coupled together with knowledge of the dispersion characteristics of plume receiving waters and dilution diffuser design will allow a more precise setting of discharge monitoring standards and licence conditions. Management and technological mitigation (such as equipment producing enhanced dilution) will be required to ensure that any hypersaline discharges are limited to the lowest tolerance of all the economically valuable species in discharge areas to avoid potential loss of revenue in associated fisheries. Finally, the findings here provide information towards a better understanding of these species' possible natural range, potential issues for laboratory husbandry and their importance in their community assemblages.

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