Avoidance by sand shrimp, *Crangon septemspinosa*, of sandy patches covered by hydrated lime (calcium hydroxide) deposits

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2011

Canadian Technical Report of Fisheries and Aquatic Sciences 2938

Fisheries and Oceans Canada Pêches et Océans Canada

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Canadian Technical Report of Fisheries and Aquatic Sciences

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Avoidance by sand shrimp, *Crangon septemspinosa*, of sandy patches covered by hydrated lime (calcium hydroxide) deposits.

by

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Cat. No. Fs 97-6/2938E ISSN (Print) 0706-6457 ISSN (Electronic) 1488-5379

Correct citation for this publication:

Reebs, S.G., Jackman, P.M., A. Locke and Fairchild, W.L. 2011. Avoidance by sand shrimp, *Crangon septemspinosa*, of sandy patches covered by hydrated lime (calcium hydroxide) deposits. Can. Tech. Rep. Fish. Aquat. Sci. 2938: iv + 7 p.

ABSTRACT

Reebs, S.G., Jackman, P.M., A. Locke and Fairchild, W.L. 2011. Avoidance by sand shrimp, *Crangon septemspinosa*, of sandy patches covered by hydrated lime (calcium hydroxide) deposits. Can. Tech. Rep. Fish. Aquat. Sci. 2938: iv + 7 p.

Individual sand shrimp were offered a choice between one side of a 6-L aquarium that featured a sandy bottom, and the other side that had the same sandy bottom covered by a layer of deposits resulting from the injection of hydrated lime, $Ca(OH)_2$, into the water column. The injection had raised water pH from 7.7 to 9.4 and had deposited a flocculent mixture of magnesium carbonate and calcium carbonate on the bottom. Shrimp significantly avoided the aquarium side with flocculent material in the first 24 h. Mortality was high during the ensuing three days (up to 75%, likely due to the raised pH), but the survivors continued to avoid the flocculent side. A control group (no lime injection) showed only a moderate mortality of 15% after 96 h and no avoidance of any particular side within the aquaria. Large quantities of concentrated lime solutions can be emptied into the sea where bio-fouling treatment is applied to mussel socks, and particles have been observed drifting to the bottom at such locations. The present results suggest that such particles can reduce the preference of sand shrimp for the affected sediment, but it remains to be seen whether flocculent deposits can persist on the seafloor in the presence of local currents, and whether sand shrimps are present in any significant number in areas where hydrated lime is used as a bio-fouling treatment.

RÉSUMÉ

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Des crevettes de sable, *Crangon septemspinosa*, furent introduites individuellement dans des aquariums de 6-l dont une des moitiés contenait un fond de sable fin tandis que l'autre contenait le même sable couvert par une mince couche de dépôts résultant de l'injection d'une solution concentrée de chaux hydratée, $Ca(OH)_2$. L'injection avait élevé le pH de 7.7 à 9.4 et déposé sur le sable un mélange de réactifs de floculation identifiés comme étant du carbonate de magnésium et du carbonate de calcium. Les crevettes ont significativement évité la zone de floculation pendant les premiers 24 h. Leur mortalité s'est élevé à 75% dans les trois jours qui suivirent (sans doute causée par l'alcalinité prononcée), mais les survivants ont continué à éviter la zone de floculation. Dans un groupe témoin (sans injection), la mortalité n'a pas dépassé 15% et les crevettes n'ont évité aucun côté de leurs aquariums. La chaux hydratée est souvent utilisée comme pesticide dans des sites d'aquaculture, et à la fin des traitements de grandes quantités de chaux sont souvent larguées à la mer. On sait que des nuages de dépôts de chaux atteignent le fond marin à de tels moments, bien qu'on ne sache pas encore si les floculants qui en résultent résistent longtemps aux courants. Si tel était le cas, les floculants représenteraient une détérioration de l'habitat pour les crevettes de sables, en autant que celles-ci soient présentes aux sites impliqués.

INTRODUCTION

Hydrated lime ($Ca(OH)_2$, also called calcium hydroxide or slaked lime) is commonly used as a pesticide by the mussel (*Mytilus edulis*) and oyster (*Crassostrea virginica*) industries on Prince Edward Island (PEI) (Locke et al. 2009). For example, mussel seed collectors are often dipped into troughs filled with solutions of hydrated lime to eliminate predatory starfish (MacNair and Smith 2000). Recently this technique has also been applied to mussel socks to rid them of invasive tunicate species. At the end of the liming process for the day the contents of the trough are often emptied into the surrounding waters (Locke et al. 2009).

Hydrated lime is a caustic agent that greatly elevates pH when dissolved in water. Though it is unstable in seawater (it readily combines with dissolved carbon dioxide to form harmless limestone, $CaCO_3$), the fact that relatively large amounts of it may be released into the environment in single locations within a bay (i.e., on aquaculture lease sites) raises concerns about its possible effects on other organisms in the environment. One such organism is the sand shrimp, *Crangon septemspinosa*, also known by the common name seven-spined bay shrimp. The sand shrimp is a small decapod crustacean that often occupies sandy habitats in shallow marine or brackish waters (Corey 1981, Sogard and Able 1991, Lazzari 2002, Locke et al. 2005). It is common all around PEI in bays that have aquaculture sites. Acute toxicity tests (Locke et al. 2009) have shown that sand shrimps can tolerate solutions of 50 mg/L of hydrated lime over 96 h (pH of this treatment varied between 8.17 and 9.12 over the duration of the experiment), but not 500 mg/L (pH 8.58-10.32). Over a longer duration of 14 days, LC_{50} for hydrated lime was found to be 53.1 mg/L corresponding to a pH ranging between 9.12-9.28 (Locke et al. 2009).

Field observations by divers have revealed that large clouds of lime particles can settle on the seafloor at sites where hydrated lime is released at the estuary surface (N. MacNair, pers. comm., in Locke et al. 2009). In the laboratory, injections of highly concentrated lime solutions in seawater aquaria can result in the formation of flocculent carpets on the substrate (pers. obs. S. Reebs). In an undisturbed aquarium such deposits are stable. In the field, where currents are likely to be present, the persistence of flocculent material is less likely but remains undetermined. The possibility exists that hydrated lime may at least temporarily foul sand shrimp habitat by creating a carpet of deposits over sandy patches, if the hydrated lime release site is in or near shallow water. Yet there is no information on the reaction of sand shrimp to such deposits. The goal of this study was to assess in the laboratory whether sand shrimp avoid sandy areas with carpets of hydrated lime deposits.

MATERIALS AND METHODS

Sand shrimp were captured on 16 November 2009 with D-frame dip nets over shallow beach habitat on the shores of Kouchibouguac Bay (46° 50.224’ N, 64° 56.036 W, GPS Map datum NAD83) in Kouchibouguac National Park, New Brunswick. Water in the shallows was 10 °C and had salinity of 6 to 8 ppt, which was below normal for the site due likely to a stronger than normal current going seaward along the beach. The sand shrimp were placed in aerated buckets filled with seawater from the collection site and transported by car to the Environment Canada Toxicology Laboratory in Moncton, NB. The animals were then placed in a single aerated tank of seawater (28 +/- 2 ppt) at a temperature of 15.4 to 16.1 °C and a pH of 7.6-7.8. Dissolved oxygen (D.O.) and temperature were measured daily and 80% of the water in the tank was renewed daily. The sand shrimps were fed frozen brine shrimp (*Artemia sp.*) (15-20 g) daily until the start of the experiment on 23 November 2009. At this time they were 2.0-3.5 cm in total length.

Sixty 6-L aquaria, 29.5 x 12.5 x 17 cm (L x W x H), were filled with seawater to a height of 15 cm above a 1.5-cm layer of fine sand, resulting in a final water volume of about 5 L. Water temperature was 15 °C, while pH was 7.71 – 7.75. Overhead fluorescent lights provided a light-dark cycle of 16:8 h, 100-500 lux at aquarium level. The 60 aquaria were divided into three groups of 20 each: Control = no liming; Early = sand shrimp placed in the aquaria immediately after liming; Late = sand shrimp placed in the aquaria 24 h after liming. Positions of the aquaria from one group were interspersed with aquaria from the other groups throughout the room.

A removable glass partition was placed mid-point along the length of each aquarium to divide them into two sides. For each of 40 aquaria a side was chosen at random and 20 mL of a 50,000 mg/L solution of hydrated lime (Havelock Lime Co., Havelock, NB, dissolved in seawater) was carefully injected into this side, which was then labelled as the limed side. The injections resulted in a 400 mg/L concentration of lime within the limed halves. For the remaining 20 aquaria, a side was also selected at random but no injection took place; however, the selected side was still labelled as having been “limed” for observations and served as the Control group.

Thirty minutes after the injections, when all particulates had settled to the bottom of the limed aquaria and formed a uniform carpet of white flocculent material over the entire limed side, the glass partition was slowly removed for all aquaria. An individual sand shrimp was then poured from the side, at the midpoint where the partition had been, into each of the 20 control aquaria, and into 20 of the 40 limed aquaria to constitute the Early group. No sand shrimp was poured into the last 20 limed aquaria, which were reserved for the Late group.

Thirty minutes later, the position (Over = above the limed area; Away = above the non-limed area) and the behaviour (buried in sand; lying or slowly moving over the bottom; swimming in the water column) of each sand shrimp was noted through instantaneous sampling. Swimming individuals were not assigned a position, as they often moved from side to side. Water pH was measured. These observations and measurements were repeated 3 h later, for a total of two samplings on the first day of the experiment.

The next day, an individual sand shrimp was poured into each of the 20 aquaria of the Late group, again from the side and at the midpoint where the partition had been. Position and behaviour of these sand shrimp, as well as all shrimp introduced the day before, were noted after 30 min and then after 4 h, for a total of two samplings that day. Shrimp that were found dead were not assigned a position. Water pH was measured. Thereafter, position and behaviour of all shrimp, along with water pH, were noted once a day for the next three days. This duration was chosen to determine if any potential avoidance would persist beyond the initial reaction, in case the animals could habituate to the flocculent material.

The nature of the flocculent material deposited by the injection of lime was analysed via ICP-OES (inductively coupled plasma – optical emission spectrometer), following pre-washing and digestion in distilled water.

RESULTS

The flocculent carpets were identified as being a mixture of magnesium carbonate (80-85%) and calcium carbonate (15-20%). They were several millimeters thick and remained intact through the five days of the experiment, though tracks sometimes appeared on them, left by the sand shrimp as they travelled over the substrate.

Sand shrimp from the Control group either swam or lay on sand during the first 3 h after being introduced into the aquaria. During the days that followed, they were either lying on sand (about a third) or buried (the remaining two-thirds) (Fig. 1), fairly evenly distributed between the two sides of the aquarium in the so-called "limed" and "non-limed" areas (in actuality, neither side was limed). On average, 47% of the 15-18 non-swimming individuals were found over the so-called "limed" area ($P > 0.05$, NS on a binomial test against a random distribution of 50%). Mortality rose from 5% in the first 24 h to 15% by the end of 96 h. Water pH in the Control group remained between 7.7 and 7.8 throughout the experiment.

Shrimp from the Early group mostly lay on sand away from the limed side during the first 3 h post-introduction and post-liming. Sixteen of 18 non-swimming individuals were found over the non-limed side ($P = 0.0007$ on a binomial test). The next day, 60% of the shrimps were dead, and mortality reached 75% by the end of the 96-h observation period. The survivors tended to stay away from the limed side. On average, only 25% of the 5-8 survivors were found over the limed area. This small percentage was consistent with the avoidance of the limed areas shown at the beginning of the experiment, but on a binomial test it was not significantly different from chance level, probably because of poor statistical power due to the small number of survivors. Water pH in this group decreased from 9.4 after lime injection to 8.7 by the end of the 96-h period.

Shrimp from the Late group were either swimming or lying on sand away from the limed area after being introduced into their aquaria, 24 h after the injection of the lime. Four hours after introduction, 15 of the 19 non-swimming individuals were found on the non-limed side of the aquaria ($P = 0.001$ on a binomial test). Mortality was 30% the next day, and it rose to 66% by the end of the experiment two days later. Most of the survivors tended to be found away from the limed side. On average, only 18% of the 9-14 survivors were found on the limed side of the aquaria. This percentage is significantly different from chance level on a binomial test ($P < 0.05$). Water pH decreased from 9.0, when the shrimps were introduced on the day after liming, to 8.8 by the end of the experiment.

DISCUSSION

Relatively high mortality of sand shrimps exposed to hydrated lime resulting in pH values between 8.7 and 9.4 is consistent with the toxicity tests of Locke et al. (2009), who found good survival at hydrated lime concentrations associated with pH ranging from 8.17 to 9.12 but complete mortality at pH 8.58-10.32 at the end of 96-h exposures. Field observations in a 2.5 m^3 mesocosm in a PEI estuary indicated increases from an initial pH in bottom water of about 8.7 to greater than pH of 10.5 about ten minutes after the addition of 0.5 kg hydrated lime (A. Locke, unpublished data). Mortality unfortunately decreased sample sizes during the later parts of the experiment, but avoidance of flocculent areas by the shrimps was clearly expressed in the early days before mortality, and continued to be expressed by survivors, who thus showed no habituation to it. We infer that sand covered by flocculent material coming from lime deposits would constitute a suboptimal habitat for sand shrimp as compared to bare sand.

The import of this result with regards to the dumping of lime solutions at bio-fouling treatment sites, near aquaculture installations, depends on several caveats. First, are sand shrimp present at such sites? In the Magdalen Islands, QC, sand shrimp was the second most common macrobenthic species recorded under mussel aquaculture sites, and was found as frequently under mussel lines as on sandy substrates away from mussel lines (Clynick et al. 2008). It is considered a typical shallow-water species in PEI estuaries (Hughes and Thomas 1971), where it has been observed (but not quantified) on and near mussel aquaculture sites (D'Amours et al. 2008). The species is abundant on sandy bottoms in shallow waters, but it can be found in deeper water and on other bottom types (Whiteley 1948). In shallow vegetated and unvegetated sites in the Gulf of Maine, it was found to be a habitat generalist (Sogard and Able 1991, Lazzari 2002). Sand shrimp were present at all channel and near-shore sites sampled within the Kouchibouguac River estuary, NB, in waters ranging from 12 to 28 °C and from salinities ranging between 4 and 32 ppt (Locke et al. 2005). It is therefore likely that sand shrimps are present at bio-fouling treatment sites in most PEI estuaries.

Second, any negative effect observed in the field is unlikely to come from elevated pH. Readings of pH in the field 1 m away from settling clouds of lime particles are always less than 8.5 (Locke et al. 2009), and there is no chemical process that could result in long-lasting high pH near the layer of flocculent material that settles at the bottom. This raises the question of whether the avoidance of flocculent material seen in the present experiment, where shrimp were chronically stressed by high pH, would also be expressed by shrimp exposed to flocculent material at normal pH levels. The answer must await further experiments.

Third, it seems unlikely that flocculent layers could persist on the seafloor *in situ*, given that bottom currents would likely sweep them away. Any disturbance to sand shrimp habitat would likely be temporary, or limited to low energy micro-habitats. A short summary of pertinent literature on hydrated lime and quicklime (calcium oxide) particle dissipation in sea water is available in Locke et al. (2009).

A peculiar feature of the results is that avoidance of the limed sides by shrimp was rapid when the deposits were fresh (the Early group showed strong avoidance within 30 min of being introduced in the aquaria) but not so much when the deposits were 24 h old (the Late group showed the same reaction as the Control group 30 min after being introduced, though they were avoiding the limed sides as much as the Early group had by 4 h later; see Figure 1). Something about fresh deposits may increase their unpleasantness, though what this factor might be remains unknown.

ACKNOWLEDGMENTS

We thank J. Mark Hanson and Ken Doe for comments and suggestions on the study, as well as Art Cook for the chemical analysis of the flocculent material. Financial support was provided by Fisheries and Oceans Canada (Gulf Region, to WLF), Environment Canada (Atlantic Region, to PMJ), and the Natural Sciences and Engineering Research Council of Canada (to SGR). Parks Canada's Kouchibouguac National Park provided access for collecting sand shrimp from a clean reference site and Jacqueline Arsenault and Ken Doe assisted in collection and transport.

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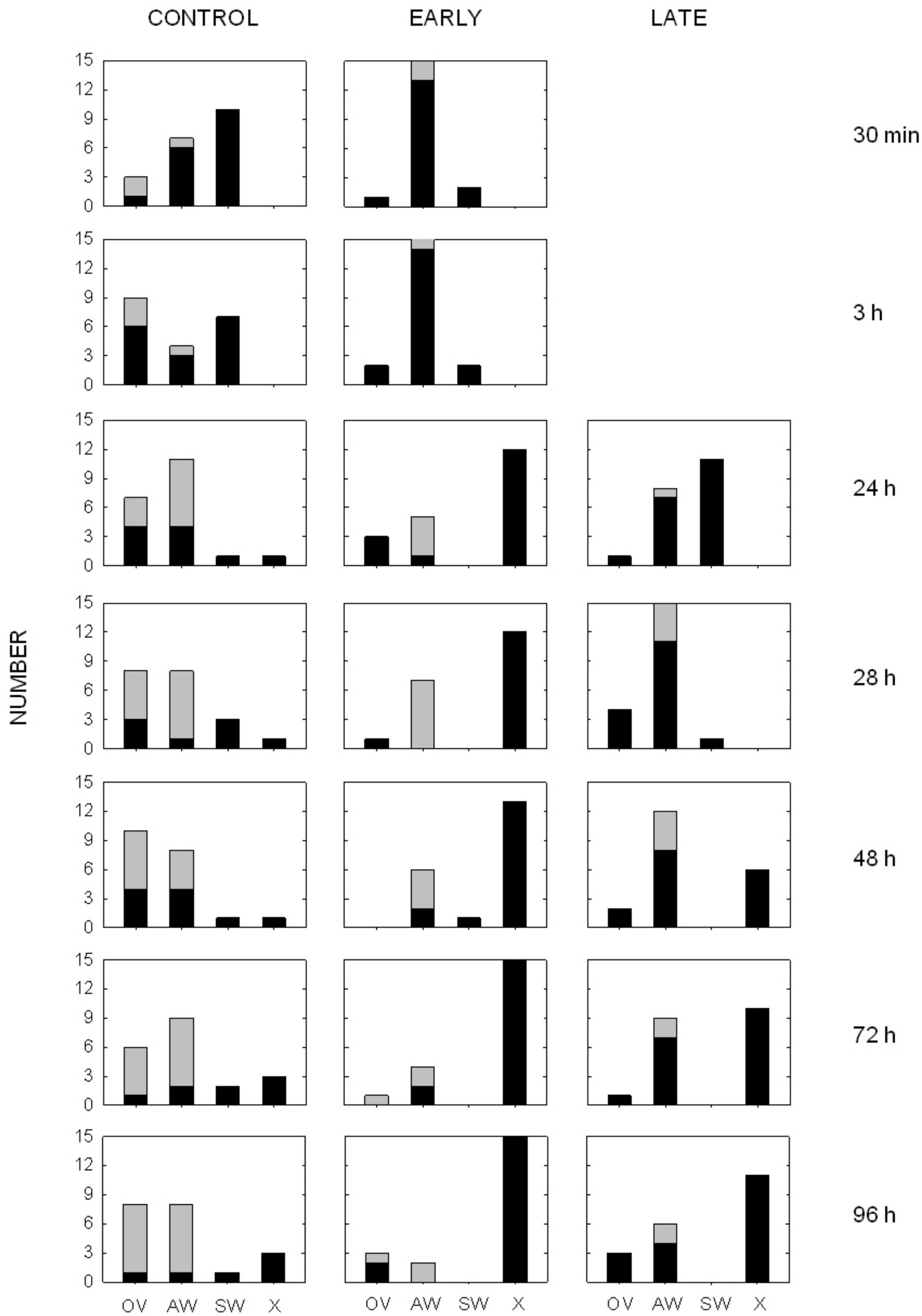

Figure 1 . Frequency distribution of sand shrimps in each group over time. OV = Over the limed area; AW = Away from the limed area; SW = Swimming; X = Dead. Grey = buried; black = on sand.