Environmental tolerance of : implications for its distribution as a marine non-native species

Gail V. Ashton, Kate J. Willis, Michael T. Burrows, Elizabeth J. Cook

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1 **Environmental tolerance of *Caprella mutica*: implications for its distribution as a**

2 **marine non-native species**

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4 Gail V. Ashton, Kate J. Willis*, Michael T. Burrows, Elizabeth J. Cook

5

6 Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Oban,

7 Argyll, PA37 1QA, Scotland

8

9 *Corresponding author:

10 Kate Willis

11 Scottish Association for Marine Science

12 Dunstaffnage Marine Laboratory

13 Oban, Argyll, PA37 1QA

14 Scotland

15

16 Tel: +44 1631 559313

17 Fax: +44 1631 559001

18 Email address: kjw@sams.ac.uk

19

20

20 **Abstract**

21 Physiological tolerances limit the distribution of marine species, with geographical

22 ranges being set by environmental factors, such as temperature and salinity, which

23 affect the rates of vital processes and survival of marine ectotherms. The

24 physiological tolerances of the non-native marine amphipod *Caprella mutica* were

25 investigated in laboratory experiments. Adult *C. mutica* were collected from a fish

26 farm on the west coast of Scotland and exposed to a range of temperatures and

27 salinities for 48 h. *C. mutica* were tolerant of a broad range of temperature and salinity

28 conditions, with 100% mortality at 30 °C (48 h LT_{50}, 28.3 ± 0.4 °C), and salinities

29 lower than 16 (48 h LC_{50}, 18.7 ± 0.2). Although lethargic at low temperatures (2 °C),

30 no mortality was observed, and the species is known to survive at temperatures as low

31 as -1.8 °C. The upper LC_{50} was greater than the highest salinity tested (40), thus it is

32 unlikely that salinity will limit the distribution of *C. mutica* in open coastal waters.

33 However, the species will be excluded from brackish water environments such as the

34 heads of sea lochs or estuaries. The physiological tolerances of *C. mutica* are beyond

35 the physical conditions experienced in its native or introduced range and are thus

36 unlikely to be the primary factors limiting its present distribution and future spread.

37

38 **Keywords**

41

2

41 **Introduction**

42 The marine amphipod *Caprella mutica*, a native of north-east Asia, is now widely

43 distributed in the northern hemisphere between latitudes 35˚N and 70˚N, representing

44 a considerable extension to its native latitudinal range (Ashton *et al.*, in press). The

45 non-native populations discovered to date are restricted to temperate oceanic coasts

46 that experience temperatures ranging from 0 to 22 ˚C, similar to that of the native

47 habitat, which has an annual seawater temperature range of -1.8 to 25 °C

48 (Schevchenko *et al.*, 2004).

49 Environmental factors are likely to play an important role in influencing the present

50 and future global distribution of *C. mutica*, as physiological tolerances result in ranges

51 being set by physical characteristics that affect the rates of vital processes and survival

52 (Vermeij, 1996). Temperature and salinity are often considered to be the principle

53 variables affecting growth, reproduction and survival of marine ectotherms (Kinne,

54 1970a; Kinne, 1970b), and can act singly or in combination to influence distribution

55 patterns (Wiederholm, 1987; Stauffer & Boltz, 1994). Previous work has shown that

56 while temperature does not limit the latitudinal distribution of *C. mutica* on the west

57 coast of Scotland (Ashton *et al.*, in press), it does influence global distribution

58 patterns of caprellid amphipods (Laubitz, 1970; Thiel *et al.*, 2003). For example,

59 temperature is the primary factor limiting the distribution of caprellids in Australasia

60 and Southern Chile (Thiel *et al.*, 2003), and in the North Pacific (Laubitz, 1970), with

61 most species occurring in the 10 to 15 °C temperature range, and only a few in the

62 sub-antarctic 5 to 10 °C range. The relatively disjunctive non-native distribution of

63 *C. mutica* within the region experiencing temperatures between 0 to 22 °C, suggests

64 that either range expansion is still underway, or factors other than temperature are

65 affecting its establishment at a global scale.

3

66   A wide salinity tolerance is typical of many amphipod species (Kinne, 1970a), with

67   several caprellid species displaying tolerance to salinities lower than 15 (Takeuchi *et*

68   *al.*, 2003). Upper salinity tolerance limits are unlikely to be a limiting factor, as

69   illustrated by Takeuchi *et al.* (2003), who found the salinity tolerance of four *Caprella*

70   spp. to be greater than 34. In the native habitat of *C. mutica*, salinities range from 11

71   to 35 (Schevchenko *et al.*, 2004). The presence of *C. mutica* in Scottish sea lochs that,

72   at times, receive considerable freshwater inputs (Ashton *et al.*, in press), suggests that

73   this caprellid is relatively tolerant of low salinities.

74   Tolerance of a wide range of environmental conditions is suggested as a characteristic

75   of successful non-native species (Van der Velde *et al.*, 1998; Strasser, 1999).

76   However, it is currently unknown whether the environmental conditions experienced

77   by *C. mutica* throughout its distribution range correspond with its environmental

78   tolerances. The caprellid may be limited to its current range by its physiological

79   tolerance limits to temperature and salinity, or other factors may be limiting its

80   biogeographic distribution. Here, we present the results of a study to investigate the

81   lethal salinity and temperature tolerance limits of the non-native amphipod *C. mutica*

82   on the west coast of Scotland. This study aimed to establish the physiological

83   tolerances of the species to these two key environmental variables that are likely to be

84   important in limiting the caprellids global distribution.

85

86   **Methods**

87   *Caprellid collection and maintenance*

88   *Caprella mutica* were collected from filamentous red algae attached to pontoon

89   structures at Dunstaffnage fish farm (56° 27.090' N 05° 27.733' W) in June 2005 and

90   2006. The animals were transported in sea water, still attached to the algae, in 10 L

91   buckets. Animals were acclimatised to laboratory conditions for 48 h at a constant

92   temperature (14 ±1 °C, ambient temperature) at a photoperiod of 8 D:16 L, the

93   photoperiod regime was maintained throughout the tolerance experiments. The

94   buckets received constant aeration and no additional food was given.

95

96   *Temperature tolerance experiments*

97   Fifteen containers (1.5 L) were filled with filtered sea water (100 μm; salinity ~35)

98   and five pieces of 5 mm mesh (150 cm^2) were added to each container. Larger,

99   healthy-looking animals were carefully isolated from the algae using forceps and

100 transferred to the mesh in the experimental containers. A maximum of 34 individuals,

101 including at least five male and five female *C. mutica* were added to each container.

102 Starting numbers in each container were recorded. Experimental containers were then

103 transferred into water baths (60 x 30 cm) maintained at five temperatures, with three

104 replicate containers at each temperature. The containers reached test temperatures

105 within 10 minutes of the start of the experiment. Temperatures were maintained using

106 either TropicMarin heaters, or a Grant chiller, and were monitored throughout the

107 experiment with temperature data loggers (Tinytag Aquatic, TG-3100). After 48 h, the

108 containers were removed from the water baths and animals were immediately

109 inspected for mortality, defined as a lack of response to mechanical stimulus (gentle

110 prodding with a blunt probe).

111 A preliminary range-finding test using temperatures of 2, 10, 22, 31 and 40°C was

112 undertaken to define approximately the lethal tolerance limits of *C. mutica*. These

113 temperatures were selected pragmatically in an effort to include mortality rates

114 ranging from 0% to 100%. In the range finding test, caprellids exposed to 40 °C died

5

115   within 5 minutes, while at 2 $^{\circ}$C they were immobile after 48 h, and lethargic when

116   stimulated. The second definitive experiment used a narrower range of temperatures

117   (21, 24, 26, 28 and 30 $^{\circ}$C) encompassing the upper lethal limit, which was estimated

118   to lie between 22 and 31 $^{\circ}$C in the range finding test.

119

120   *Salinity tolerance experiments*

121   Experimental procedures and conditions were as described for the temperature

122   tolerance tests, with the exception that the salinity tolerance experiments were

123   maintained at a temperature of 14 ±1 $^{\circ}$C in a constant temperature room.

124   Large healthy-looking caprellids were transferred to exposure tanks (1.5 L) containing

125   filtered sea water (100 μm) at five different salinities. Salinities were achieved by

126   addition of artificial sea salt (Tropical Marin) to water treated by reverse osmosis.

127   Salinity was confirmed using a portable refractometer (Atago, S/MILL) at the

128   beginning and end of the 48 h exposure periods. After 48 h, individuals were

129   inspected for mortality by gentle prodding with a blunt probe.

130   As above, a preliminary range-finding test was done using salinities of 0, 10, 20, 30

131   and 40 in an attempt to incorporate concentrations causing 0% and 100% mortality. In

132   the range finding test, caprellids exposed to a salinity of 0 were immobile within 5

133   minutes, with 100% mortality at salinities of 0 and 10 after 48 h. The upper lethal

134   limit was higher than 40 (<10% mortality at salinities of 30 and 40). The second

135   definitive experiment employed a narrower range of salinities (9, 13, 15, 18 and 20)

136   encompassing the lower lethal limit, which was estimated to lie between 10 and 20 in

137   the range finding test.

138

139 *Statistical Analysis*

140 Median lethal LT_{50} and LC_{50} values and associated 95% confidence limits were

141 calculated using probit (Finney, 1971) or trimmed Spearman-Karber analysis (TSK;

142 Hamilton *et al.*, 1977; U.S.E.P.A., 1990) using MINITAB. MINITAB uses a Chi-

143 squared analysis (χ^2) to test whether the data fits the Probit model. When χ^2 rejected

144 the Probit model ($P > 0.05$), a trimmed Spearman-Karber analysis was used to

145 calculate a non-parametric estimate of the lethal value. A disadvantage of the TSK

146 analysis is that it only yields the median lethal response (LC_{50}) and 95% confidence

147 limits. When the test results are not unimodal or symmetrical about the median lethal

148 response, the confidence intervals (CI) can be unreliable. A pairwise students' *t*-test

149 was used to compare differences in mortality of male and female *C. mutica* at each

150 temperature.

151

152 **Results**

153 *Temperature tolerance*

154 Following 48 h exposure to temperatures between 21 $^{\circ}$C and 30 $^{\circ}$C, there was a

155 marked increase in mortality of *C. mutica* at temperatures greater than 26 $^{\circ}$C, with

156 100% mortality at 30 ℃ (Figure 1). At 24 $^{\circ}$C, mortality was increased (~25%) relative

157 to that at 26 $^{\circ}$C, and similar to mortality at 28 $^{\circ}$C. The reason for this is unknown, but

158 may be a response to other uncontrolled experimental conditions (e.g. pH or oxygen).

159 Mortality was <10% at temperatures of 21 $^{\circ}$C and 26 $^{\circ}$C. The 48 h LT_{50} (± 95% C.L.)

160 was 28.3 ± 0.4 ℃. There was no significant difference in mortality between male and

161 female *C. mutica*.

162

7

163   *Salinity tolerance*

164   100% mortality was observed following 48 h exposure of *C. mutica* to salinities lower

165   than 15 (Figure 2). At a salinity of 18, individuals were lethargic and less responsive

166   to stimulation than those at 20. The lower 48 h LC_{50} ($\pm$ 95% C.L.) was 18.7 $\pm$ 0.2. The

167   lower LC_{50} for females (18.0 $\pm$ 0.3) was significantly higher ($P<0.05$) than that for

168   males (19.3 $\pm$ 0.4).

169

170   **Discussion**

171   Adult *C. mutica* collected on the west coast of Scotland were tolerant of a wide range

172   of temperature and salinity conditions in 48 h laboratory exposures. The upper

173   thermal tolerance limit of *C. mutica* (48 h LT_{50}, 28.3 °C) was higher than maximum

174   temperatures experienced in its non-native Scottish habitat (16 °C; Ashton *et al.*, in

175   press), or in its native habitat (25 °C, Schevchenko *et al.*, 2004). The lower lethal

176   temperature was not determined as there was no mortality at the lowest temperature

177   tested (2 °C), although the caprellids were immobile after 48 h at 2 °C and were

178   lethargic when stimulated. After 48 h at a salinity of 18, *C. mutica* were lethargic, and

179   at salinities lower than 16, 100% mortality was observed. These salinity values are

180   also higher than those recorded in the native and non-native habitat of the caprellid

181   (11 and 10.5 respectively; Schevchenko *et al.*, 2004; Ashton *et al.*, in press).

182   The current experiments were run for a period of 48 hours, and therefore only give an

183   indication of the short-term tolerance of *C. mutica.* However, the thermal tolerance

184   limits recorded in this study suggest that *C. mutica* could survive introduction to the

185   tropics or the poles, extending its current distribution. In the northern hemisphere, *C.*

186   *mutica* has been introduced to both coasts of North America, from 37.5 to 48.5 °N and

187   to the Atlantic coasts of Europe from 49.5 to 69.1 $^{\circ}$N. In the southern hemisphere, *C.*

188   *mutica* has only been described from New Zealand (Ashton *et al.*, in press). Sea

189   surface temperatures at the equator are relatively stable at 26 to 27 $^{\circ}$C throughout the

190   year (Tait, 2001), slightly lower than the 48 h upper lethal limit of 28 $^{\circ}$C. However,

191   the ability of *C. mutica* to survive prolonged exposure to high temperatures is

192   unknown, and it is also possible that earlier life stages may not be as tolerant to high

193   temperatures as the adults.

194   In its native habitat, populations of *C. mutica* survive at temperatures as low as -

195   1.8 $^{\circ}$C, and non-native populations have established in areas that freeze at the surface

196   during winter, such as Passammaquoddy Bay, Canada (Ashton *et al.*, in press). Given

197   the lethargic state of the caprellids after 48 h at 2 $^{\circ}$C in the present study, it is likely

198   that growth and reproduction are reduced, or cease, at such low winter temperatures.

199   The salinity tolerance of adult *C. mutica* is similar to or slightly less than that of other

200   *Caprella* species. The lower 48 h LC_{50} (18.7 ± 0.2) is higher than that of *C. scaura*

201   and *C. equilibria*, with 24 h LC_{50} values of 7.5 and 11.5 respectively (Cockman &

202   Albone, 1987), noting that these values are for 24 h exposures, not 48 h. For other

203   *Caprella* species, *C. danilevski*, *C. subinermis*, *C. penantis* and *C. verrucosa*, 48 h

204   LC_{50} values ranged from 12 to 17 (Takeuchi *et al.*, 2003). The increased salinity

205   tolerance of male *C. mutica* in the present study may reflect the sexual dimorphism of

206   the species (the males are considerably larger) or differences between the sexes in

207   resource allocation to reproduction (Hoback & Barnhart, 1996). It is surprising,

208   however, that this trend was not observed with temperature, which might be expected

209   to produce a greater physiological response (Davenport & Davenport, 2005).

210   In the native habitat, *C. mutica* individuals experience salinities as low as 11

211   (Schevchenko *et al.*, 2004), considerably lower than the 48 h LC_{50} in this study. Thus,

212   the current realized distribution of *C. mutica* with respect to salinity is beyond its

213   fundamental niche described here. This is contrary to the expectation for marine

214   ectotherms (Sprague, 1963; Tait, 2001). In coastal habitats, *C. mutica* are probably

215   able to survive fluctuating salinity conditions during the tidal cycle and short periods

216   of exposure to low salinity during periods of heavy rainfall. However, prolonged

217   exposure to salinity lower than 18 is likely to adversely affect the caprellid, and may

218   explain why populations are absent from the upper reaches of Scottish sea lochs that

219   regularly experience low salinities (Ashton pers. obs.).

220   The broad physiological tolerance of adult *C. mutica* would suggest that changing

221   temperature and salinity conditions experienced during transport through equatorial or

222   polar regions would be unlikely to adversely affect individuals in ballast water (Witt

223   *et al.,* 1997) or on ships' hulls (Minchin and Gollasch, 2003). Species distributions

224   that are limited by thermal tolerances are expected to change in response to climate

225   change (e.g. marine mammals, Tynan & DeMaster, 1997; coral reefs, Hoegh-

226   Guldberg, 1999; pelagic ecosystems, Boyd & Doney, 2002). Predicted rates of range

227   expansion in terrestrial environments include 6.1 km per decade (Parmesan & Yohe,

228   2003) and 12.5 - 24.8 km per decade (Hickling *et al.*, 2006). In particular, evidence

229   suggests that many invasive species possess traits that will enable them to capitalize

230   on the various elements of global change, exacerbating the impacts of invasions on

231   stressed ecosystems (Dukes & Mooney, 1999). Climate change will be important in

232   determining the future distribution of *C. mutica*, which can be expected to extend

233   polewards.

234   In addition to environmental conditions, the success of non-native species is also

235   influenced by the frequency of introduction events, which are generally more

236   common in temperate waters (Minchin & Gollasch, 2003; Drake & Lodge, 2004)

237 compared to tropical or polar regions, propagule pressure (the frequency and size of

238 introduction events), and biotic interactions in the recipient region such as

239 competition, facilitation and predation (Bruno *et al.*, 2005). Thus, the current

240 distribution of *C. mutica* is likely to reflect a combination of introduction

241 opportunities, as well as favourable abiotic and biotic conditions in these areas.

242 Broad physiological tolerance and plasticity are commonly cited as characteristics of

243 successful invading species (Van der Velde *et al.*, 1998), with temperature and

244 salinity tolerances often combining to limit the potential distributions of marine

245 species (Wiederholm, 1987; Stauffer & Boltz, 1994). Given the broad temperature

246 and salinity tolerance of adult *C. mutica* observed in this study, the caprellid is likely

247 to be limited to marine habitats experiencing a temperate climate (not exceeding

248 26 °C), and regions where salinity does not drop below 18 for extended periods of

249 time. Evidence suggests that dispersal via human vectors is increasing the distribution

250 of *C. mutica* at a faster rate than climate change (Ashton, 2006). Therefore, at present

251 human dispersal will be the most important factor in determining the range expansion

252 of *C. mutica*.

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348

349   **Figure Legends**

350

351   Figure 1. Mortality of *C. mutica* following 48 h exposure to temperatures between 21

352   and 30 ºC (mean ± S.E., n = 3). Reference lines indicate 48 h LT_{50} ± 95% CI

353   (broken lines) estimated using TSK analysis.

354

355   Figure 2. Mortality of *C. mutica* following 48 h exposure to salinities between 9 and

356   20 (mean ± S.E., n = 3). LC indicates lethal concentration of percentile

357   mortalities; the broken reference line indicates the lower 48 h LC_{50} (estimated
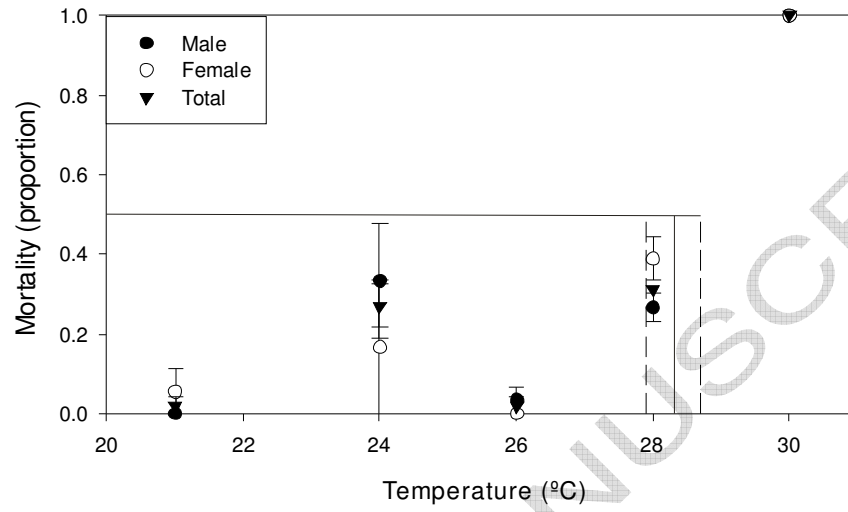
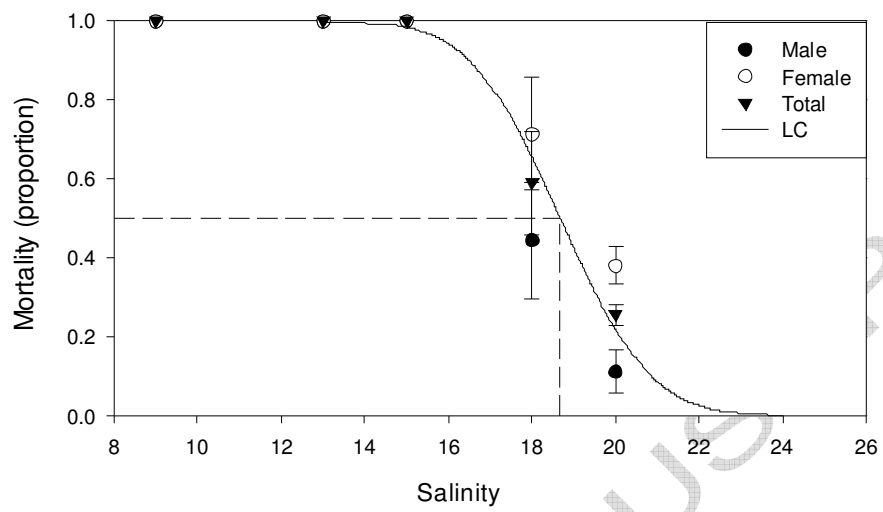358   using Probit analysis).

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361   Figure 1.

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