Title

Tonic immobility in a marine isopod: the effects of body size, sex, and colour morph

Running head

Tonic immobility in *Cleantiella isopus*

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Abstract

Tonic immobility is considered an anti-predator defence, wherein prey adopts a motionless state in a characteristic posture elicited by external stimuli. The marine isopod *Cleantiella isopus* exhibits tonic immobility with an arch-like posture and motionless state lasting several seconds or minutes in response to external stimuli such as predatory attacks by fish. In this study, we describe tonic immobility by wild-caught *C. isopus* and examine the influence of body size, sex, and colour morph on the frequency and duration of tonic immobility. All individuals exhibited tonic immobility regardless of body size, sex, or colour morph, suggesting that the behaviour plays a major role in predator avoidance following detection by a predator. In males, smaller individuals exhibited more prolonged tonic immobility than larger individuals, whereas the relationship between the duration of tonic immobility and body size was unclear in females. Colour morph had no effect on the duration of tonic immobility. These findings provide a detailed documentation of tonic immobility in *C. isopus* and suggest that the factors affecting tonic immobility differ between males and females.

Keywords: anti-predator behaviour, body size, death feigning, marine isopod, thanatosis, tonic immobility

Introduction

Tonic immobility, also known as death-feigning or thanatosis, is a behavioural trait in which prey adopt a motionless state in a characteristic posture elicited by external stimuli (Humphreys & Ruxton, 2018; Sakai, 2021). Several hypotheses have been proposed to explain the adaptive value of this behaviour (Miyatake *et al*., 2004, 2009; Ruxton, 2006). For example, it can increase survival after detection by visual predators that are better able to detect moving prey, as these predators are less likely to attack immobile prey (Miyatake *et al*., 2004, 2009). Some of the adaptive value of this behaviour is specific to certain predator types; for example, grasshoppers adopt a rigid posture during tonic immobility to avoid being swallowed whole by gape-limited predators (Honma *et al*., 2006).

Although tonic immobility has been documented in many terrestrial taxa, including both vertebrates and invertebrates (Humphreys & Ruxton, 2018), there are relatively few studies that have quantified tonic immobility in marine invertebrates. Behaviour resembling tonic immobility is frequently documented in decapod crustaceans in both freshwater and marine environments (Mackay, 1929; Powell & Gunter, 1968; O'Brien & Dunlap, 1975; Field, 1990; Bergey & Weis, 2006; Scarton *et al.*, 2009; Coutinho *et al*., 2013). Tonic immobility in decapod crustaceans has been documented primarily in species targeted by bird and fish predators, and its occurrence as a response

against nemertean predators has also been observed (Christy *et al.*, 1997; Hazlett & McLay, 2000; Bergey & Weis, 2006).

The frequency and duration of tonic immobility can be influenced by individual characteristics such as physiological context (Miyatake, 2001a; Krams *et al.*, 2013), sex (Miyatake, 2001a, b; Kuriwada *et al.*, 2009), and body size (Hozumi & Miyatake, 2005; Farkas, 2016). However, these influences are not consistent across taxa. For example, whereas increased body size is associated with prolonged tonic immobility in a weevil (Hozumi & Miyatake, 2005), small body size is associated with more frequent tonic immobility in a stick insect (Farkas, 2016). Similarly, females exhibit prolonged tonic immobility in comparison to males in a seed beetle (Miyatake *et al.*, 2008), but a shorter period of immobility in a leaf beetle (Mueller & Mueller, 2017). In the marine system, the duration of tonic immobility by the rock crab *Heterozius rotundifrons* varies with chemical and visual cues (Field, 1990; Hazlett & McLay, 2000, 2005), but is not significantly affected by body size or sex (Bach & Hazlett, 2010).

Marine isopods are targeted by various predators including flatworms, crabs, birds, and fish in intertidal and subtidal zones (Wallerstein and Brusca, 1982; Leidenberger *et al.*, 2012). They possess various defensive traits, including microhabitat choice (Merilaita & Jormalainen, 1997; Vesakoski *et al.*, 2008), chemical defence

75 (Lindquist *et al.*, 2005), and cryptic colouration (Merilaita, 1998; Hultgren & Mittelstaedt,
76 2015). Sexual differences in predator avoidance response are well-studied in *Idotea*
77 *baltica* (Jormalainen & Tuomi, 1989; Merilaita & Jormalainen, 1997; Vesakoski *et al.*,
78 2008). *Idotea baltica* females reduce their activity during reproduction in the face of high
79 predation risk, whereas males retain high activity despite the risk because they prioritise
80 mate search over predator avoidance (Jormalainen *et al*., 1989; Vesakoski *et al.*, 2008).
81 This higher risk aversion in females relative to males is thought to reflect differences in
82 their strategies for maximizing reproductive success (Vesakoski *et al.*, 2008).

83 The marine isopod *Cleantiella isopus* is a common intertidal benthic species
84 found under rocks and on algae on the intertidal rocky shore. *Cleantiella isopus* has a
85 lifespan of 13–15 months from birth (at a body size of about 5 mm) to death. In our study
86 area, they usually have three breeding periods: from late February to mid-April and from
87 late May to early June, with some individuals breeding again in July (Takahashi &
88 Goshima, 2012). This species exhibits tonic immobility with an arch-like posture and
89 motionless state in response to external stimuli such as attacks by predatory fish (Figure
90 1). To our knowledge, no studies have previously documented tonic immobility in aquatic
91 isopods, although some reports exist for terrestrial isopods (Quadros *et al.*, 2012; Tuf *et*
92 *al.*, 2015; Cazzolla Gatti *et al.*, 2020). In this paper, we describe the tonic immobility of

C. isopus and examine the influence of body size, sex, and colour morph on the frequency and duration of tonic immobility.

Materials and Methods

Study animals

The population of *C. isopus* used in this study consists of five distinct colour morphs which can be classified as brown, brown with a white line, brown with a white spot, light brown, and green (Takahashi & Goshima, 2012). Three of the brown-based morphs appear to be well concealed in brown algae such as *Neorhodomela aculeata*, whereas the light brown and green morphs are well concealed on sandy substratum. Patterns of microhabitat utilization are similar among the colour morphs and between the two sexes, since these categories often coexist within the same shelter under stones during low tide (Takahashi & Goshima, 2012).

Individuals were collected from an intertidal rocky shore in Kattoshi (41°44′N, 140°36′E) on the western side of Hakodate Bay, southern Hokkaido, Japan, during low tides in April and May 2022, the breeding season of *C. isopus* (Takahashi & Goshima, 2012). In total, we collected 230 individuals, including 159 females and 71 males, with body length ranging from 14.1 mm to 36.7 mm. They were brought back to the

111 laboratory and individually placed in a small plastic container (130 × 95 × 65 mm, L ×
112 W × H) filled with natural seawater (approximately 30 mm in depth) at ambient water
113 temperature (approximately 13 °C). The following observations were conducted after an
114 acclimation period of one hour. After the experiment, all individuals were released back
115 into the field site from which they were collected.
116
117 **Observation of tonic immobility**
118 The procedure for the behavioural trials was based on previous studies of terrestrial
119 isopods (Quadros *et al.*, 2012). Tonic immobility was induced by poking each isopod
120 five times with a stick to simulate an attack by a predatory fish. This stimulus was
121 specifically intended to imitate immobilization by intertidal fish species (e.g.,
122 *Chaenogobius annularis*, *Pholis crassispina*), which are common predators in our study
123 area. Preliminary observations indicated that the posture shown during tonic immobility
124 prevents the isopods from being swallowed whole by fish, leading to repeated attacks
125 from fish attempting to swallow them (Supplementary Video S1). If the isopods in our
126 behavioural trials responded to the five consecutive pokes by adopting the posture
127 associated with tonic immobility (Figure 1), the duration of immobility was recorded
128 using a stopwatch. The duration was defined as the time elapsed between the end of the

129 stimulation and the time when the individual moved slightly, which typically began with
130 a movement of the second antennae. If the individual did not exhibit tonic immobility,
131 the result was recorded as “no response”. Because the duration of tonic immobility in
132 preliminary observations was mostly between 60 and 120 s, we observed the behaviour
133 of each isopod for 300 s. Each individual was tested three times, with each test
134 separated by approximately 30 min.

135 Body length, sex, and colour morph were recorded after the third behavioural
136 trial. Body length was measured as the distance from the anterior border of the cephalon
137 to the posterior border of the telson (Takahashi & Goshima, 2012; Miura & Goshima,
138 2016). The sex of each individual was identified by using a stereo microscope on the
139 basis of the presence (male) or absence (female) of genital papillae on the underside of
140 the pleon. The experiments were conducted in the early part of the breeding season, and
141 all females in our study had brood pouches containing embryos in an early stage of
142 development (Takahashi & Goshima, 2012).

143

144 **Statistical analysis**

145 We constructed a generalized linear mixed model (GLMM) with gamma error
146 distribution and log link function for the duration of tonic immobility. Individual ID was

included as a random effect in the model to account for nonindependence among the three trials for each individual. We included body length, sex (male or female), and colour morph (brown, brown with a white line, brown with a white spot, light brown, or green) as fixed effects. We included all possible interaction terms in the full model, but since the interaction terms were not significant, we did not include them in the final model. The significance of fixed effects was tested by Wald χ^2 tests. As males and females differed in body length, all data were segregated by sex (males, $n = 71$; females, $n = 159$). All statistical analyses were performed in R (version 4.2.1; R Core Team, 2022). Models were fitted using the *glmmTMB* function from the package *glmmTMB* (version 1.1.5; Brooks *et al.*, 2017). Fixed effects were tested using the *Anova* function from the package *car* (version 3.1.1; Fox & Weisberg, 2019).

Results

All individuals ($n = 230$) exhibited tonic immobility in all trials, and the duration of tonic immobility was greater than 300 s in 19 out of the 690 total trials. We observed two distinct postures during tonic immobility: one in which the second antennae are extended (Figure 2A) and another in which the second antennae are folded (Figure 2B).

There was a significant effect of body size on the duration of tonic immobility

165 ($df = 1$, $\chi^2 = 37.305$, $P < 0.001$; Table 1). Although a negative relationship was found
166 between the duration of tonic immobility and body size (*Estimate* = –0.047 ± 0.008 SE;
167 Figure 3), it depended on sex. The negative relationship was clear in males but not in
168 females (Table 1; Figure 3). There was no significant effect of colour morph on the
169 duration of tonic immobility ($df = 4$, $\chi^2 = 7.955$, $P = 0.116$; Table 1).
170
171 **Discussion**
172 All individuals exhibited tonic immobility, which is particularly impressive given the
173 large number of individuals examined in this study. The high frequency of this
174 behaviour implies that tonic immobility is a major response against predatory attack in
175 *C. isopus* regardless of body size, sex, or colour morph. The arch-like posture adopted
176 during tonic immobility may be an adaptation to predators that swallow their prey
177 whole (Moore & Williams, 1990; Honma et al., 2006). The high frequency of tonic
178 immobility observed in this study could indicate a high lethality of predator attacks
179 when isopods fail to assume the correct posture. Based on preliminary observations, we
180 used five pokes with a stick as a stimulus to induce tonic immobility, but this stimulus
181 may have been unnatural. Excessively strong stimulation could have caused the high
182 frequency of tonic immobility observed in our study, but we believe this is unlikely

because no individuals appeared to have been injured by the stimulation during the trials. Instead, we suggest that the uniformity of our stimulation method explains the uniformity of the observed response.

We found a negative relationship between overall body size and the duration of tonic immobility. This pattern was particularly evident in males. Male body size is quite variable (Takahashi & Goshima, 2012), and this variation might mean that there are large differences in predation risk among male *C. isopus*. In contrast, the range of female body size was small, indicating that factors other than body size are likely to be responsible for the observed variation in the duration of tonic immobility. We hypothesise that the negative relationship between the duration of tonic immobility and body size may reflect a decrease in predation risk with increasing body size. If this were the case, smaller individuals could derive a greater benefit from remaining immobile for longer periods. Smaller individuals may also be more elusive to a visual predator since they can become concealed among fragments of seaweed during periods of tonic immobility. A similar mechanism has been hypothesized for a stick insect (Farkas, 2016), where smaller individuals that exhibit tonic immobility are thought to have a greater chance of avoiding detection by a predator after falling to the ground. It is also possible that small and large *C. isopus* are preyed upon by predators of differing size

and/or type. Several types of predators, including flatworms, crabs, and fish, are present in our study area (K. Igarashi, personal observation). Based on the hypotheses outlined above, we believe that tonic immobility is a specific behavioural defence against predation by fish that rely on visual cues for prey detection. In addition to being visual predators, most fish are gape limited, meaning they swallow their prey whole. The role of tonic immobility in isopods appears to encompass both immobility (which inhibits visual detection) and posture (which inhibits ingestion by gape-limited predators). However, further research is required to fully understand these two aspects.

A similar pattern of decreasing predator avoidance response with greater body size has been demonstrated in other marine invertebrates. For example, the American lobster *Homarus americanus* exhibits ontogenetic changes in its preferred microhabitats, preferring sheltering habitats early in life but expanding to an increasingly wide range of habitats as it grows (Wahle, 1992). Among *Idotea*, interference competition for microhabitats is influenced strongly by body size, with small males more likely than large males to leave a crowded habitat (Franke *et al*., 2007). If small males are forced to use habitats that are not protected from predators, they may exhibit a longer duration of tonic immobility to cope with the heightened predation risk. Nevertheless, this specific variation in habitat use has not been

219 documented in *C. isopus*, and individuals in this study were all collected from the same
220 habitat (under rocks) during low tide. However, it remains possible that microhabitat
221 usage in this species differs by body size or sex during other tidal or diurnal periods.
222 Populations of *C. isopus* comprise individuals of various ages, reflecting the
223 species' lifespan of 13–15 months and its three synchronized reproductive cycles
224 (Takahashi & Goshima, 2012). Thus, if the large individuals in our study were older
225 than the small ones, our result may partially reflect an ontogenetic shift toward shorter
226 tonic immobility in *C. isopus*. Larger individuals are at a more advanced developmental
227 stage, and in males, there is a greater tendency to initiate precopulatory guarding during
228 the breeding season (Jormalainen & Merilaita, 1995; Miura & Goshima, 2016), which
229 may result in a decreased response to predation risk. During the breeding season, *I.*
230 *baltica* males exhibit increased activity and have been documented to appear more
231 reckless to predators (Jormalainen & Tuomi, 1989; Vesakoski *et al*., 2008).
232 The lack of a discernible relationship between the duration of tonic immobility
233 and body size in females might be attributed to a temporary behavioural restriction in
234 ovigerous females. As in other isopods, *C. isopus* females incubate their embryos in the
235 ventral brood pouch, and all females in our study had brood pouches containing
236 embryos in an early stage of development (Takahashi & Goshima, 2012). In the

terrestrial isopod *Armadillidium vulgare*, rolling behaviour is an effective anti-predator defence to protect the vulnerable ventral part, but ovigerous females have difficulty maintaining a rolled-up posture due to the presence of the brood pouch (Suzuki & Futami, 2018). Ovigerous females might also have difficulties exhibiting tonic immobility due to the mechanical constraints of the brood pouch. Females that hold developed embryos in the brood pouch may instead rely on primary defences such as reducing activity to avoid detection by predators (Jormalainen & Tuomi, 1989). However, the lack of variation in the reproductive status and developmental stage of the embryos in our data hinders a comprehensive evaluation of this hypothesis.

There was no significant effect of colour morph on tonic immobility in our study. Tonic immobility often functions as a secondary defence, meaning it occurs after detection by predators (Endler, 1986). This could explain the lack of a strong relationship with colour morph, which is likely to function as a primary defence (i.e., it works prior to detection by predators). Alternatively, the lack of effect of colour morph could simply reflect the artificial conditions and uniform background colours used in our behavioural trials. Further studies on natural substrata that allow for effective concealment of each morph may reveal an interaction between colour morph and the duration of tonic immobility.

255 Our results partially explain variations in tonic immobility in the marine isopod
256 *C. isopus*. However, our data do not demonstrate whether this behaviour is effective for
257 avoiding predation and are not sufficient to identify which types of predators induce it.
258 The effectiveness of anti-predator defences can depend on the relative speed of
259 movement of predators and prey (Aguilera *et al.,* 2019). Tonic immobility might be
260 advantageous for prey species that are extremely slow-moving in comparison to
261 predators, meaning that fleeing is ineffective. Numerous studies in well-studied artificial
262 selection systems have reported a negative relationship between locomotor performance
263 and selection for tonic immobility (Ohno & Miyatake, 2007; Miyatake *et al.*, 2008;
264 Nakayama *et al.*, 2010, 2012; Matsumura *et al.*, 2016). Hazlett *et al.* (2000) studied
265 predator avoidance among five sympatric decapod species and documented immobile
266 behaviour resembling tonic immobility in two of the less mobile species. Further studies
267 are needed to determine whether this pattern applies more widely. Tonic immobility has
268 also been observed in other Idoteidae, e.g., *C. strasseni* and *I. ochotensis* (K. Igarashi,
269 personal observation). Understanding the taxonomic prevalence of tonic immobility and
270 the predators that induce it will provide a general understanding of this behavioural
271 defence in marine crustaceans. Some marine invertebrates (e.g., hermit crabs or
272 gastropods) take refuge in shells and remain quiescent for several minutes. Although

this behaviour resembles tonic immobility in some respects, it may be best to classify this as a distinct behaviour.

Data availability

The datasets for this study are available from the corresponding author upon reasonable request.

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Author contributions

Koichi Igarashi: Conceptualization, Methodology, Formal analysis, Investigation, Writing–original draft, Writing–review & editing, Visualization. **Satoshi Wada**: Writing–original draft, Writing–review & editing, Supervision.

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Conflict of interest

The authors declare none.

Ethical standards

The experiments conducted as part of this study, which involved crustaceans, did not require approval from the Hokkaido University Animal Experiment Committee, as per their guidelines. Consequently, no specific approval number was assigned. However, we made concerted efforts to minimize the distress experienced by the study animals throughout the course of the experiments.

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Figure legends

Fig. 1. Examples of *Cleantiella isopus* posture (A) during tonic immobility and (B) after tonic immobility. The ruler in the background is in units of centimetres.

Fig. 2. Two distinct postures of *Cleantiella isopus* were observed during tonic immobility: with the second antennae (A) extended or (B) folded. The scale bar is in units of centimetres.

Fig. 3. Relationships between body size of *Cleantiella isopus* and the duration of tonic immobility for males (A) and females (B).

467 **Tables**

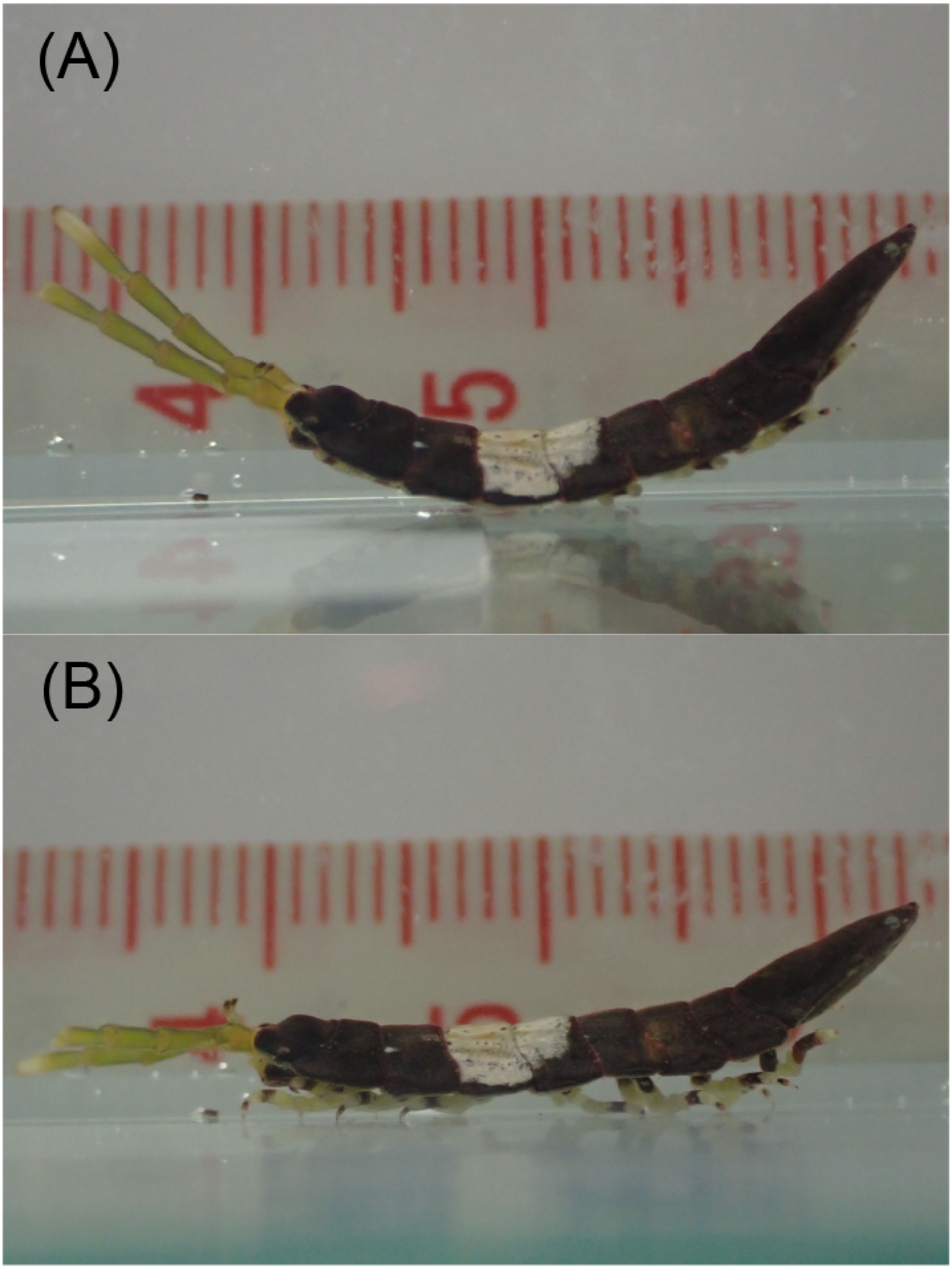
468 Table 1 GLMM analysis of the duration of tonic immobility in *Cleantiella isopus* using

469 gamma error distribution and log link function. Significant effects (at $P \leq 0.05$) are

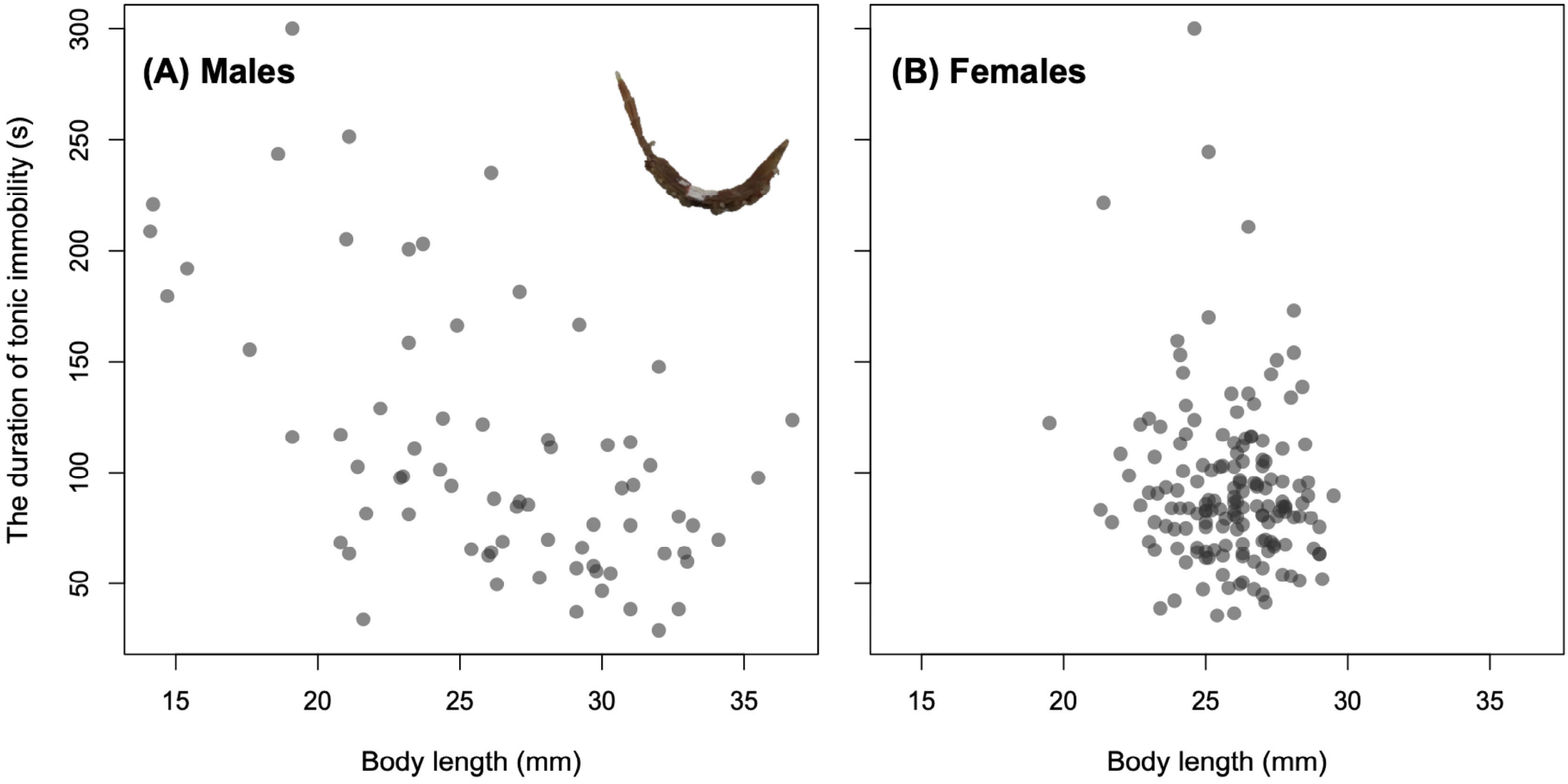
470 shown in bold.

	df	χ^2	*P*
All isopods			
Body size	**1**	**37.305**	**<0.001**
Sex	**1**	**7.397**	**0.005**
Colour morph	4	7.955	0.116
(A) Males only			
Body size	**1**	**24.387**	**<0.001**
Colour morph	4	4.640	0.326
(B) Females only			
Body size	1	3.524	0.060
Colour morph	4	6.747	0.150

471


(A)
(B)


(A)
(B)


(A) Males
(B) Females
The duration of tonic immobility (s)
50
100
150
200
250
300
15
20
25
30
35
Body length (mm)
Body length (mm)