1   **Changes in metabolic rate of spiny lobster under predation risk**

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5   **Running page head**: Lobster metabolic rates under predation risk

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191. Abstract

20 Animals exposed to high levels of predation risk may exhibit a variety of changes to life

21 history, behaviour, physiology and morphology that can affect survival. Under predation

22 threat, prey individuals may increase their aerobic metabolism to allocate energy toward

23 escaping behaviours (e.g. 'fight-or-flight'), although the associated energetic cost of such

24 behaviour remains largely unknown. Lobsters display different anti-predatory responses such

25 as sheltering and/or escaping, but the underlying energetic cost of such responses has not

26 been examined. Here, we tested the aerobic, metabolic response of lobsters (*Jasus edwardsii*)

27 in the presence of predator (octopus, *Octopus maorum*) olfactory cues (kairomones) using

28 open-flow respirometry. We examined the routine metabolic rate of lobsters in response to

29 predator kairomones during the active phase of their diurnal cycle (at night) to examine the

30 physiological anti-predator response when lobsters are most vulnerable. Our findings

31 revealed that lobsters strongly reduced their routine metabolism for three hours by 31.4 %

32 when exposed to kairomones in comparison to controls. Our findings suggest that under

33 laboratory conditions, lobsters exposed to predation risk during the night reduce their activity

34 to avoid predators; i.e., the anti-predator mechanism is 'to be immobile or inactive' rather

35 than a 'fight-or-flight' response. Lobster immobility may be an energetically advantageous

36 predation response in the short term, however prolonged or regular predator exposure could

37 have significant consequences on foraging time and foraging area in turn, with an overall

38 impact on lobster performance particularly in environments with high predator presence such

39 as fishing grounds.

40 Key words

41 Predation risk; Fight-or-flight, aerobic metabolism, kairomones, *Jasus edwardsii*, octopus

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2. Introduction

Predation can play a key role in the ecological and evolutionary dynamics of populations, as well as in the structure and dynamics of food webs, communities and ecosystems (Murdoch et al. 2003). Early Population ecology theory has generally conceived of predation as a functional response (e.g. predator searching for and consuming prey) based on the dynamics of the relative density of the prey population, assuming prey as individuals to be unresponsive entities (e.g. Murdoch & Oaten 1975). Over the last decades, however, a large body of evidence suggests that prey is anything but unresponsive (Sih 1985; Lima & Dill 1990; Preisser et al. 2005). Predation risk, or the non-consumptive effects (NCEs), that a predator might exert on its prey can strongly alter key prey traits such as behaviour, morphology, life history and physiology, exerting selective pressures with evolutionary implications (Lima & Dill 1990; Werner & Peacor 2003; Hawlena & Schmitz 2010; Sheriff & Thaler 2014). These NCEs can have severe, even fatal impacts on prey (Preisser et al. 2005), affecting prey demography (e.g. Zanette et al. 2014), community structure and ecosystem processes (Hawlena & Schmitz 2010;Trussell & Schmitz 2012).

Predation risk can alter organismal physiological nutrient balances by inducing energetically costly stress responses involving hormonal, cellular and metabolic processes (Hawlena & Schmitz 2010). The general paradigm for predation stress responses (reviewed by Hawlena & Schmitz 2010b) involves increased levels of glucocorticosteroids (hormonal) and heat shock proteins (cellular) resulting in increased cardiorespiratory activity and aerobic metabolic rate (Slos & Stoks 2008). The increase in aerobic metabolism is associated with the 'fight-or-flight' response (e.g. Slos & Stoks 2008) as a consequence of a redirection of energy to locomotory structures to allow an enhanced ability to escape predators (Hawlena & Schmitz

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66 2010b). These stress responses can have substantial consequences for prey at different time

67 scales, from short term impacts on acute survival, to long-term responses such as inhibition

68 of development and reproduction, slower growth rate and reduced body condition, and

69 reduced assimilation efficiency (conversion of food into body tissue) (Hawlena & Schmitz

70 2010b). While our current knowledge of prey stress responses under predation risk mostly

71 comes from terrestrial and freshwater predator–prey systems, very little is known about the

72 physiological response of prey under predation risk in the marine realm (Trussell et al. 2006;

73 Matassa & Trussell, 2014).

74 In activating physiological mechanisms associated with anti-predator responses, prey must

75 first evaluate the level of risk and the benefit of defence (Kats & Dill 1998; Cooke et al.

76 2003). 'Sensing' for predator risk reduces uncertainty in prey decision making (Sih 1992) and

77 many animals assess chemical cues from the environment (Kats & Dill 1998) to detect and

78 evade predators (Ferrari et al. 2010). Aquatic crustaceans may rely strongly on water-borne

79 chemicals for assessing predation risk, as is the case for spiny lobsters which can avoid

80 potential predators by detecting chemical cues from key predators such as octopus (e.g.

81 Berger & Butler 2001; Gristina et al. 2011; Buscaino et al. 2011), or alarm cues from injured

82 or killed conspecifics (Shabani et al. 2008; Briones-Fourzán 2009; Hazlett 2011).

83 The risk allocation hypothesis suggests that prey adaptively allocate their foraging efforts and

84 therefore their exposure to predation across high-risk and low-risk situations (Lima &

85 Bednekoff 1999). Additionally, the spatial component in such decision making by prey (e.g.

86 food or shelter) can also involve the selection of areas for foraging (e.g. broad-scale habitat

87 vs patchy habitat) (Sih 1992). For example, feeding rates are higher in open habitats

88 compared with patchy areas where refuges are more abundant, although they represent highly

89 dangerous areas with increased predation risk (Sih 1992). In lobsters, sheltering behaviour

90   appears to be an evolutionary trait with predation risk as one of the most important factors

91   altering shelter occupancy (e.g. Weiss et al. 2008).

92   Predators can directly interact with fisheries, generating mortality by consuming organisms

93   from the fishing gear during capture, known as 'depredation' (Uhlmann & Broadhurst 2013).

94   Trap- or pot-based fishing activities for lobsters enhance their predation risk because these

95   activities can reduce the lobster's capacity to escape predators and the act of fishing can also

96   attract key predators such as octopus. The southern rock lobster *Jasus edwardsii* is highly

97   exposed to predation risk by the Maori octopus *Octopus maorum* on fishing grounds in

98   southern Australia (Brock & Ward 2004; Hunter et al. 2005; Harrington et al. 2006; Briceño

99   et al. 2015; Briceño et al. 2016) and New Zealand (Ritchie, 1972). Octopus hunting strategy

100   is more effective when lobsters are in confined spaces (e.g. natural shelters) (Bouwma &

101   Herrnkind 2009) and consequently very successful on individuals confined in fishing traps

102   (Brock & Ward 2004). Additionally, octopus also consume the bait within lobster traps or

103   pots in some fisheries (e.g. South Australia, Brock et al. 2003; South Africa, Groeneveld et

104   al. 2006) demonstrating the habituation of octopus to the lobster fishery. It is presently

105   unclear how octopus abundance and predation activity on the fishing grounds can modify key

106   lobster life history traits such as growth, feeding, and reproduction.

107   High-predation-risk environments can generate chronic stress responses to predation risk

108   resulting in reduced assimilation efficiency (e.g. Trussel et al. 2006), decreases in production

109   (growth and reproduction) and altered body nutrient content (Hawlena & Schmitz 2010).

110   Examining the energetic cost of responses to predation risk (e.g. Cooke et al. 2003) is,

111   therefore, a fundamental step in evaluating the stress physiology of lobsters under predation

112   risk. Moreover, quantifying the energetic costs associated with predation will allow a better

113   understanding of how changes in aerobic metabolism affect prey at an individual level

114 (Cooke et al. 2003), and propagate through to the population level and ultimately, the

115 function of the associated ecosystem (Hawlena & Schmitz 2010; Zanette et al. 2014).

116 Spiny lobsters are generally nocturnal foragers, remaining inside their shelter during the day

117 and foraging outside the shelter at night, resulting in a basic circadian pattern in most lobster

118 species (MacDarmind et al. 1991; Weiss et al. 2008). However, circadian patterns can be

119 modulated by different intrinsic (e.g. mating behaviour and molting cycle, Childress & Jury

120 2006) and extrinsic factors (e.g. intense sunlight and moon phase, Childress & Jury 2006)

121 with predation risk being one of the most important selective forces in lobster shelter

122 occupancy (Weiss et al. 2008). In some spiny lobsters, elevated predation risk is able to drive

123 diurnal foraging behavior, shelter choice, and gregariousness (see Loflen et al. 2010; Withy-

124 Allen et al. 2013; Berriman et al. 2015). Previous research has shown a strong circadian

125 rhythm in lobster metabolic rates, which correlates with activity, demonstrating a strong

126 relationship between behaviour (e.g. movement) and energy expenditure (Crear & Forteath

127 2000). Understanding of the physiological responses of prey individuals under predation risk

128 requires consideration of the circadian rhythm in metabolic rates as anti-predator responses

129 can largely differ between day and night in crustaceans (see Sakamoto et al. 2006). Studies

130 on aquatic physiological ecology have played an important role in linking individual-based

131 energetic traits (e.g metabolic rates) with predator-prey traits (e.g escaping behaviour),

132 demonstrating how lab-based experimental physiology can assist to address ecological

133 questions with implications for fishery and conservation management (Cooke et al. 2013;

134 Cooke et al. 2014; Hollins et al. 2018).

135 In this study, we hypothesize that lobsters under predation risk increase their metabolic rates

136 according to the general stress paradigm (Hawlena & Schmitz 2010). First, we examined how

137 lobster routine metabolic rate (RMR) changes throughout the circadian cycle. This

138 experiment was considered as a base line to define a suitable period to add kairomones from

139 octopus, taking into account when lobster aerobic metabolism would be highest. We then

140 recreated scenarios of predation risk during the night (high activity, nocturnal scenario) in

141 which lobster RMR was examined under presence and absence of octopus odour or

142 kairomones. The current study represents the first attempt to define aerobic metabolic

143 changes associated with anti-predator responses at individual levels in lobsters.

144

145 3. Materials and Methods

146 3.1   Animal collection

147 A total of 25 inter-moult adult *Jasus edwardsii* were collected in a scientific reserve with an

148 area of approximately 1 km^2 at Crayfish Point near Hobart in Tasmania, Australia (42°57.2'S

149 : 147°21.2'E). Lobster collection was performed by lobster traps in February (2014), and

150 individuals of both genders measuring up to 110 mm of carapace length (minimal legal size

151 for fishing) were used. In addition, Maori octopus (*Octopus maorum*) individuals (3–6 kg; n

152 = 3) were collected as by-catch from the same trapping survey. Lobsters and octopus were

153 maintained at the aquaculture facilities of the Institute for Marine Antarctic Studies,

154 University of Tasmania, Hobart, Australia. Lobsters were separated by gender into two

155 rectangular tanks (1,900 L, 2.22 m x 2.05 m x 0.93 m), and provided with hollow concrete

156 building blocks (15 per tank) as shelters. Lobsters were fed with live mussels (*Mytilus*

157 *galloprovincialis*) three times per week to satiation. Octopus were individually placed in 800-

158 L circular tanks with artificial shelters. Tanks were covered with black mesh to avoid

159 escaping behaviour, and octopus were fed with prawns (*Fenneropenaeus merguiensis*) daily

160   to satiation. Lobsters and octopus were kept at natural ambient temperature ($16.5 \pm 1°C$) and

161   salinity at 35 ± 1 ‰ with a natural light cycle over two weeks before starting the

162   experiments. Octopus collection, maintenance and handling were conducted under the

163   University of Tasmania Animal Ethics Committee, permit approval No. A0013584.

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165   3.2   Respirometry

166   The rate of oxygen consumption was measured using an intermittent open flow respirometry

167   system as described in detail by Jensen et al. (2013a). Two 3.55-L respirometric chambers

168   (radius: 48 mm; length: 480 mm) were immersed in a 455-L tank ('bath') to ensure

169   temperature stability. Each chamber contained an oyster mesh (5-mm mesh size) fitted to the

170   lower section to provide a tractional surface as suggested for crustacean respirometry (Dall

171   1986). Dissolved oxygen was recorded every 10 s using a luminescent dissolved oxygen

172   optode (Hach LDP, HQ40d, Hach company, USA). Two submersible aquarium pumps

173   (Quietone 1200) were connected to each chamber. One pump was used to mix the water

174   inside the chamber and to deliver water past the oxygen optode at a rate of 1.0 exchange min^{-1}

175   (3.55 L min^{-1}) (closing cycle). The other pump was intermittently exchanging water

176   between the inside of the chamber and the outside of the chamber at a rate of 1.0 exchange

177   min^{-1} (flushing cycle). A flushing cycle was performed every 10 minutes using a digital timer

178   (DRT-1, Sentinel, China). This resulted in measurements of oxygen consumption every 10

179   minutes (i.e. 6 measurements h^{-1}) that were averaged to provide hourly means for analysis.

180   Respirometry chambers were carefully rinsed with fresh water after each trial, and sterilized

181   with chlorine after every second trial. During $M\dot{O_2}$ measurement, oxygen levels never fell

182   below 90% saturation to avoid inducing a hypoxic stress response by the lobsters (Jensen et

183   al. 2013a). Background oxygen consumption was measured in empty chambers after each

8

184 trial for two to four hours as described in Jensen et al. (2013a). Lobsters were fasted for 72

185 hours before any measurement to generate a similar post-prandial state among individuals

186 (Jensen et al. 2013a). All respirometry trials were undertaken with two lobsters that were

187 individually and randomly placed into one of the two respirometric chambers, with an

188 acclimation period of 6 hours approximately. Data from this period was not included for the

189 analyses.

190

191 3.3   Circadian patterns in lobster routine metabolism

192 Changes in in routine metabolic rates (RMR) throughout the circadian pattern of lobster

193 activity were measured in inter-moult adults of both sexes (n = 16; 514–732 g) under a

194 natural light cycle from 12:12 to 10:14 hours of light: dark cycle during March–April (2015).

195 We define the diurnal phase as the time between 06:00 and 18:00 (twilight), and the nocturnal

196 phase as between 18:00 to 06:00 (dawn). The circadian pattern of lobster RMR was examined

197 for 24 hours with trials starting approximately at 12 pm, with each trial examining two

198 lobsters only used once.

199 RMR was differentiated between night and day as 'RMRn' and 'RMRd' respectively.

200 Additionally, the standard metabolic rate (SMR) was calculated as the mean of the lowest

201 10% of measured values following Fitzgibbon et al. (2014a).

202

203 3.4   Lobster routine metabolism under nocturnal predation risk scenarios

204 The effect of predation risk on lobster routine metabolism during the night was examined

205 using predator odour as a kairomone (inter-species chemical cues). This experiment was

206 undertaken using exclusively males (n = 10; 461–769 g) between June and July 2014. The

9

207   exclusion of females was due to disparity of the moulting cycle between the sexes (Ziegler et

208   al. 2004), which is accompanied by profound physiological changes (Fitzgibbon et al.

209   2014b).

210   Water temperature was maintained the same as the circadian pattern experiment, ($16 \pm 1°C$)

211   using a titanium heater (2000 W, 8.3 A). Three male octopus (4–6 kg each) were randomly

212   used for the trials; the same individual was not used in consecutive trials. In addition, a red

213   light was used to observe lobsters over the nocturnal periods- these light conditions did not

214   alter the circadian rhythm in other lobster species (e.g. *Panulirus argus*, Weiss et al. 2006),

215   such as has been for *in situ* behavioural observations of *J. edwardsii* (Mills et al. 2005).

216

217   3.5   Experimental system

218   We used an experimental set up that consisted of three main units (Fig. 1): the conditioning

219   tank (unit A), the treatment tank (unit B) and the respirometric chambers (unit C). Units B

220   and C were described in the respirometry subsection (3.2). Unit A was a circular tank (180 L)

221   used to prepare octopus kairomones. This tank was supplied with mechanically filtered water

222   from an open-flow water system (50 μ cartridge). Unit A was placed approximately 60 cm

223   above unit B, and both units were connected with a PVC tube (50 mm diameter). The water

224   outlet from B was directly discarded allowing an open-water circulation from A to B. In

225   addition, water from the bath was incorporated into the respirometric chambers during the

226   flushing cycles previously described in the respirometry section (3.2).

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230 3.5.1   Protocol and experimental design

231 To depict the predation risk scenarios under night conditions we developed the following

232 protocol (Fig. 1):

233 Step 1- Lobster acclimation: Two lobsters were individually placed into each respirometry

234 chamber at approximately 12:00 until 18:00 (6 hours).

235 Step 2 – Kairomone concentration: The concentration of kairomone was created by holding

236 an octopus in the conditioning tank for one hour without water exchange. A 1 h concentration

237 period was used to standardize the accumulation of octopus kairomones without resulting in a

238 deterioration of such cues as recently demonstrated (e.g. less than one hour, Chivers et al.

239 2013). Additionally, the concentration period was suitable to minimize octopus excretion that

240 also may have altered our results, as predator faeces can also act as kairomones (Ferrari et al.

241 2010). To ensure the lack of predator faeces, octopus were deprived of food for two days

242 prior to experimentation. Furthermore, water condition (i.e. temperature and dissolved

243 oxygen) was monitored in the conditioning tank, where the dissolved oxygen was kept at

244 saturation level (>90%) using an air stone. After one hour, the octopus was gently removed

245 from unit A and placed back in the maintenance tank.

246 Step 3 – Kairomone exposure: The water from unit A containing the kairomones was directly

247 flushed into the bath during the last 2-3 minutes of the flushing cycle of the respirometer,

248 recreating an acute kairomone exposure. After flushing, the conditioning tank was

249 immediately, thoroughly rinsed with fresh water. The open-flow water system was then

250 reconnected allowing the new sea water to flow from unit A to unit B but with no octopus in

251 it.

252   By using this protocol we assume that (1) lobsters were acutely exposed to octopus

253   kairomones and (2) such exposure was gradually weakened as cues were diluted over the

254   time after reconnecting the open-flow water circulation (Step 3, Fig. 2). Considering the

255   water exchange in the bath, it was estimated that kairomones remained within this unit for

256   less than 3 hours.

257   Lobster routine metabolic rates were examined during 48-hour trials over two nights (night 1

258   and night 2, Fig. 2). Each night we applied one of the two predation risk scenarios: the

259   scenario under predation risk (treatment) or the scenario without predation risk (control) with

260   the order of these scenarios randomised among consecutive trials. The protocol for the

261   control experiments were the same as previously described for the predation risk treatment

262   but with the absence of kairomones. Each octopus was used more than once, although not in

263   consecutive trials. Lobsters were used only once.

264   Under the presence of kairomones, RMRn after kairomone exposure (KE) was differentiated

265   as 'RMRnrisk'. RMRnrisk and was recorded until the end of the nocturnal period of

266   respirometry (06:00), resulting in a period of seventy six hours after KE.

267

268   3.6   Data analysis

269   Metabolic rates and background respiration were determined by linear regressions of the rate

270   of decline in dissolved oxygen concentration for every 10 minutes over the closing cycle.

271   Individual animal measurements were averaged to present hourly means in order to account

272   for temporal variation in recorded $M\dot{O}_2$ resulting from spontaneous activity which is a well-

273   known component of animal routine metabolism.

274   Temporal changes in routine metabolism were analysed using generalized linear mixed

275   models (GLMMs), which could account for the lack of independence in repeated oxygen

276   measurements (Zuur et al., 2009). Normality of residuals was assessed by visual inspection

277   (e.g histogram of model residuals), and homogeneity of variance was tested by Bartlett's test

278   for normal distribution of data (Sokal and Rohlf, 1995). The circadian pattern of RMR was

279   examined by a GLMM including the period (day/night) as a fixed effect and individuals as a

280   random effect. Differences in RMR between predation risk scenarios were examined by

281   including predation risk scenarios (absence/presence of kairomones) and time (e.g. hours KE)

282   as an interaction term (predation risk * time), as well as the order of treatments among nights

283   as fixed effects and individual as a random effect. Additionally, the significance of factors

284   was further examined by one-way ANOVA with significant differences identified by Tukey's

285   HSD tests for post-hoc multiple comparisons. The significance value used was 0.05. All

286   analyses were performed in R (v.3.4.4), using packages 'lme4' for the GLMMs and 'ls

287   means' for Tukey's HSD tests.

288 **4. Results**

289 4.1   Circadian pattern in lobster routine metabolism

290 Mean nocturnal RMR was 0.062 mg O2 h-1 g-1, significantly higher than mean diurnal RMR

291 (0.040 mg O_2 h^{-1} g^{-1}) ($F_{1,299}$ = 233.226 , p < 0.001) indicating a profound circadian pattern in

292 lobster aerobic metabolism (Fig. 3). Lobsters typically increased RMRd by 50% between

293 14:00 and 18:00 (twilight), and then remained at an average RMRn of 0.062 mg $_{O2}$ h^{-1} g^{-1}

294 until 06:00 (dawn). Additionally, RMRd rapidly decreased between 06:00 and 07:00, which

295 was quite consistent among individuals with a mean RMRd of 0.038 mg O_2 h^{-1} g^{-1}. The

296 standard metabolic rate (SMR) was 0.03 mg O_2 h^{-1} g^{-1}. Overall, RMRd and RMRn were 1.3

297 and 2.1 times higher than SMR respectively.

298

299 4.2   Routine metabolism under nocturnal predation risk

300 Analysis of nocturnal routine metabolic rate (RMRn) before kairomone exposure showed no

301 variability among individuals ($F_{1,14}$ = 0.0576, p = 0.818). Lobsters exposed to octopus

302 kairomones strongly reduced their RMRnrisk for three hours by up to 31.42 % (0.02 mg O_2 h^{-}

303 ^{1} g^{-1}) (Fig. 4), resulting in significant differences among predation risk scenarios ($F_{1,113}$ =

304 13.16, p < 0.001) as well as for the interaction term (predation risk * time) ($F_{1,113}$ = 7.423, p <

305 0.01). This response differed significantly between exposed and control lobsters at two

306 (Tukey's HSD, t = 3.148, p < 0.05) and three (Tukey's HSD, t = 3.634, p < 0.01) hours after

307 KE. Furthermore, the reduction in routine metabolism at three hours after KE resulted also in

308 a lower variability among individuals (coefficient of variation=31%) compared to controls

309 (38.2%). Between three and five hours after being exposed, animals rapidly increased their

310 RMR, reaching similar values to pre-treatment condition. Independently of the treatment,

311 RMRn was strongly reduced from five hours onwards, demonstrating the effect of time (F =

312 3.89, df = 51, p < 0.05), which was close to dawn (06:00). Considering the pattern in RMR

313 observed in treated animals, predation risk can be divided into two periods: (1) RMR

314 decrease over the first three hours, and (2) subsequent RMR recovery with a duration of two

315 hours.

316

317 5. Discussion

318 In this study we examined the effect of predator kairomones on the routine metabolism of

319 adult *Jasus edwardsii* as a proxy of the energetic cost experienced by lobsters under predation

320 risk. We report (1) the presence of a circadian pattern in lobster routine metabolic rates,

321 resulting in increasing oxygen consumption of lobsters during night time and (2) how

322 temporal changes in metabolic rates can be altered during the night under simulated predation

323 risk scenarios. Our findings demonstrate that lobsters did not increase their routine metabolic

324 rates under predation risk as we expected in our initial hypothesis, based on the general stress

325 response (Hawlena & Schmitz 2010). Instead, lobsters reduced their routine metabolism by

326 approximately 31% for up to three hours under the nocturnal kairomone exposure. Individual

327 activity within a respirometric chamber is metabolically expressed as routine metabolism,

328 hence animals that move more in the chambers consume more oxygen as largely documented

329 in crustacean respiratory physiology (Crear & Forteath 2000; Kemp et al. 2009; Toscano &

330 Monaco 2015). The decreasing RMR observed after KE would suggest that lobsters reduce

331 activity as an anti-predator mechanism (e.g. immobility), appearing as an alternative

332 hypothesis to the general stress response. We further discuss the energetic and ecological

333 implications of immobility as an avoidance predator mechanism in this lobster species,

15

334   contributing new insights into physiological mechanisms underlying decision making in prey

335   under predation risk in aquatic organisms.

336   5.1   Circadian pattern in lobster routine metabolism

337   Changes in light cycle play a crucial role in the regulation of activity in lobsters

338   demonstrating a circadian rhythm in activity (Childress & Herrnkid 1994; Weiss et al. 2008).

339   In this study, the nocturnal routine metabolic rate (RMRn) increased by approximately 50%

340   compared with the diurnal RMR (RMRd) which is similar to that reported in other lobsters

341   (*Panulirus cygnus*, Crear & Forteath 2001). Major changes in RMR were observed during

342   dusk (18:00) and dawn (06:00) as previously reported in *J. edwardsii* (Crear & Forteath

343   2000), as well as in other lobsters (*Panulirus homarus*, Kemp et al. 2009; *P. cygnus*, Crear &

344   Forteath 2001). Such changes in lobster activity throughout the light cycle have been also

345   reported in field (e.g. MacDarmind et al. 1991) and laboratory (Williams & Dean 1989)

346   studies in *J. edwardsii*. The circadian rhythm of RMR is suggested to match changes in

347   activity by lobsters within the respirometer (Crear & Forteath 2000, 2001; Kemp et al. 2009).

348   Previous studies in *J. edwardsii* have reported a strong correlation between activity and

349   metabolic rate by continuous observations from a respirometer with video cameras (Crear &

350   Forteath 2000). Although we did not perform systematic observations of lobster activity

351   within the respirometer, random observations during trials confirmed that lobsters become

352   more active during the night and inactive during the day.

353

354   5.2   Routine metabolism under a nocturnal predation risk scenario

355   Nocturnal routine metabolism of lobsters was reduced by up to 31.42 % three hours after KE

356   in comparison with controls, with RMRnrisk returning to control levels five hours after KE.

357 Our findings do not match the general premise of increased respiration rates as the first

358 physiological response of prey under predation risk. Instead, we found that threatened

359 lobsters reduced their nocturnal RMR. Similar decreases in aerobic metabolism under

360 predation risk has been reported in arachnids (e.g. Okuyama 2015), fish (Holopainen et al.

361 1997; Cooke et al. 2003) and tadpoles (Steiner & Van Buskirk 2009). Reductions in

362 metabolism and cardiovascular activity are suggested to mitigate the risk of predation,

363 reducing the need to invest in costly anti-predator responses such as escaping behaviours (e.g.

364 'flight-or-fight') (Hawlena & Schmitz 2010).

365

366 5.3   Immobility-or-flight in lobsters?

367 Predation risk can be minimized in lobsters by two major strategies (Herrnkind et al. 2001;

368 Buscaino et al. 2011): (1) 'predator-avoidance mechanisms' (e.g. sheltering, immobility and

369 nocturnal activity); and (2) 'anti-predator mechanisms' (e.g. escape, aggregation, cooperative

370 defence and weaponry). Such mechanisms, in addition, can operate sequentially as recently

371 reported in some rock lobsters (*Palinurus elephas*, Buscaino et al. 2011). For example, a

372 lobster might remain immobile to avoid detection by a predator; however, once detected it

373 may use the ultimate avoidance behavior of tail-flipping to move away from the predator and

374 towards safer areas (Mills et al. 2008; Buscaino et al. 2011). Integrating such information

375 with our results, we have demonstrated that the reduction in lobster routine metabolism under

376 predation risk confirms the immobility response as a predator avoidance mechanism in this

377 species. This can be further supported by studies using octopus as a predator model on

378 decapods in which inactivity is the most common strategy to avoid an encounter with octopus

379 (Table A1, Appendix).

380 Changes in prey activity induced by the presence of a predator's odour are well known across

381 taxa (Kats & Dill 1998), and reduction in prey activity also appears to be associated with the

382 presence of visual predators like octopus (Hanlon & Messenger 1998; Kats & Dill 1998).

383 However, recent advances in our understanding of the octopus olfactory system (e.g. *Octopus*

384 *vulgaris*, Polese et al. 2015) suggest that chemical signalling can be another source of sensory

385 input that could work in combination with visual cues or alone to provide ecological

386 information especially in light-limited habitats (Nilsson et al. 2012). The role of olfaction in

387 octopus seems to be strongly associated with reproduction (Polese et al. 2015), although

388 previous studies in octopus (Boyle 1983) and cuttlefish (Boal & Golden 1999) have

389 demonstrated that individuals exposed to food odour increases movement (e.g. arousal) and

390 ventilation rates. The olfactory capacity in octopus for prey searching indicates a potential

391 alternative hypothesis to explain the immobility response by lobsters. If *Octopus maorum* is

392 able to detect kairomones from *J. edwardsii* as a target prey, lobster immobility may rise as a

393 strategy to reduce lobster kairomone or metabolites emission in order to remain 'chemically

394 quiet'. Lobsters are hard-shelled animals that store urine and faeces, allowing them to be

395 'chemically quiet' when necessary (e.g. reproduction, Atema 1995). For example, urine

396 signals can be used during lobster dominance (e.g *Panurilus argus*, Shabani et al., 2009) and

397 courtship as chemical cues in the urine are involved with memory (Atema 1995) and

398 individuals can avoid agonistic behaviour by hiding their reproductive status by chemical

399 quiescence (e.g. Díaz & Thiel 2004).

400 Previous information on chemical ecology among conspecifics should be taken into account

401 for designing experiments to examine lobster physiological responses under predation risk. In

402 this study, each respirometry trial was undertaken with two lobster individuals that were

403 visually and chemically exposed to the same experimental conditions. *J. edwardsii* is a

404 gregarious species during sub-adult and adult stages (Butler et al. 1999), so signals between

405 individuals are biologically/ecologically relevant. The physiological response by lobsters to

406 predator exposure in the wild is also likely to include intraspecific cues. Hence the need for

407 further studies including the number of conspecifics on lobster physiology as examined in

408 fish respirometry (Herskin 1999).

409 So far there is little information about the energetic cost associated with either predator

410 avoidance or anti-predator mechanisms in lobsters. Escape response such as tail-flipping is a

411 high-cost energetic behaviour in crustaceans as large muscle fibres that facilitate tail-flipping

412 are involved (England & Baldwin 1983; Jimenez et al. 2008). The dependency on anaerobic

413 metabolism results in an oxygen debt which must be aerobically recovered (Jimenez et al.

414 2008; Jensen et al. 2013b). We suggest that the lowering of metabolic rates associated with

415 the immobility response may operate as an energetic strategy rather than investing in a costly

416 escape behaviour such as tail flipping (e.g. 'flight') (Hawlena & Schmitz 2010). Such a

417 strategy can be further examined using studies examining the excess post-exercise oxygen

418 consumption (EPOC) as proxy of energetic cost and recovery in lobsters (e.g. *Sagmariasus*

419 *verreauxi*, Jensen et al., 2013b). Lobsters required around 8.36 mg O_2 h^{-1} g^{-1} and more than 10

420 hours for recovery following tail-flipping until exhaustion, representing around 1.2 times the

421 standard metabolism needed during such recovery a period (Jensen et al. 2013b). Using these

422 values, we further explore our findings by comparing the daily energetic cost needed for

423 standard (e.g. resting) and routine metabolism (e.g. activity) in the absence of and in the

424 presence of predation risk, as well as EPOC from Jensen et al. (2013b) (using caloric

425 equivalent 1 mg O_2 g^{-1} = 14.3 J mg^{-1}; Lucas 1993) (Fig. 5).

426 According to our energy estimations, a single escaping event until exhaustion would be

427 17.5% above the energy required for nocturnal activity assuming a period of 12 hours. In

19

428   contrast, the immobility response may represent a 'saving' strategy compared with tail-

429   flipping although it would imply around 50% of the nocturnal period under inactivity. The

430   activation of both anti-predator strategies (immobility or flight) may depend on the type of

431   cues (e.g. chemical and visual) and on kairomone concentrations, as previously suggested

432   across taxa (Lima & Steury 2005),with a greater perceived risk likely resulting in a flight

433   response. Although in this study kairomones were not quantified to recreate the predation risk

434   scenarios, under the experimental conditions tested here *J. edwardsii* did not perform tail-

435   flipping as an anti-predator response. Whilst we compared both defence mechanisms from an

436   energetic point of view, such strategies are possibly equally effective in reducing the lethal

437   effect of predators, and are likely used in combination. Studies have described behavioural

438   responses of lobster-octopus interaction in an experimental arena, describing the sequence as

439   'freezing'– weaponing– tail-flipping (see Buscaino et al., 2011), which certainly validates the

440   hypothesis of immobility as a primary defensive mechanism before displaying costly

441   escaping behaviours in *J. edwardsii*. It is worth mentioning that tail-flipping can also impose

442   other challenges for lobsters as the process can move the organism to a completely unknown

443   environment and increase the exposure in the water column to predators who are reactive to

444   movement based prey.

445   Linking lab-based physiological information with behavioural traits such as prey defence

446   mechanisms can lead to context dependant responses which must be considered for future

447   studies. For example, the confined space (e.g respirometry chamber) in which lobsters were

448   exposed to predator cues could have limited the display of defensive mechanisms known in

449   lobsters (e.g tail-flipping or walking). Recent studies have suggested the relevance of

450   examining methodological and functional relationships (Careau et al. 2008) between

451   behavioural and physiological responses in an ecological context (e.g predation risk with

452 chemical cues) (e.g Toscano & Monaco 2015). In the previous study by Toscano & Monaco

453 (2015), crabs exposed to predator cues within respirometry chambers become more active

454 than crabs exposed in a mesocosm under the same predation risk. The authors attribute such

455 behavioural differences due to the lack of refuge habitat in respirometry chambers, likely

456 leading to enhanced attempts to hide or escape. Although respirometry chambers used here

457 may act as a sheltered space for threatened lobsters, it is unclear whether the same avoidance

458 response (immobility) may occur in an opened experimental set up (e.g arena). Future studies

459 examining physiological (e.g metabolic rates) responses in a predator-prey context should

460 examine such methodological relationships (e.g Toscano & Mocano 2015). However this

461 may itself create additional practical challenges as accurate oxygen consumption

462 measurements strongly rely on the restrictive methodological aspects of respirometry (e.g

463 animal size: chamber volume proportionality, Clark et al. 2013).

464 Cue concentration is an important extrinsic factor that can modulate prey anti-predator

465 responses in aquatic systems (Ferrari et al. 2010). Prey likely use kairomone concentration to

466 adjust the intensity of their antipredator response in a threat-sensitive manner (Helfman 1989)

467 to optimize fitness in the trade-off between predator avoidance responses (e.g sheltering) and

468 fitness-related activities (e.g foraging) (Ferrari et al. 2010). In this study, the predation risk

469 scenario was recreated by exposing lobsters to a single and highly concentrated octopus cue

470 during the flushing phases of respirometry for 1 hour. Additionally, it is expected that other

471 extrinsic factors such habitat type, water motion and chemistry (Ferrari et al. 2010) could also

472 play important roles in lobster chemical ecology, and should be also considered in future

473 investigations. Aggregative behaviour, size distribution of conspecifics and the availability of

474 size-structured shelters are factors that can vary predation risk in lobsters (e.g Berger &

475   Butler 2001), and their inclusion in experimental designs would be desirable to achieve a

476   more realistic background to examine lobster physiological traits under predation risks.

5.4 477   Ecological implications

478   Low-cost energetic strategies such as immobility may be used more regularly in individuals

479   from regions with high predation pressure, which are less likely to waste energy in flight

480   responses, preserving energy to cope with more extreme stress events (e.g. attack) given the

481   abundance of predators as reported in freshwater fishes (Brown et al. 2005; Gravel et al.

482   2011). Predation risk for lobsters may be elevated in fishing areas, given high abundance of

483   predators like octopus as has been demonstrated in south eastern Australia (Briceño et al.

484   2015; Briceño et al. 2016). As octopus can be attracted by lobster traps (e.g. baiting, Brock et

485   al. 2003; Phillips et al. 2012), lobster fishing may increase consumptive and non-consumptive

486   effects towards foraging lobsters. Further to direct KE emission from the abundance of

487   octopus in the fishing grounds, octopus depredation within lobster traps might also increase

488   the emission of alarm cues from injured or freshly killed conspecifics, further reducing

489   lobster activity (Shabani et al. 2008; Hazlett 2011). A reduction in activity or increase in

490   sheltering behaviour would be expected in threatened lobster individuals, negatively affecting

491   both growth and fitness as demonstrated in other marine organisms (Trussell et al. 2006;

492   Matassa & Trussell 2014). Increasing sheltering behaviours during the night may result in

493   negative effects on lobster growth as foraging rates would be reduced.

494   Studies have demonstrated that size selectivity by a fishery (e.g targeting larger individuals)

495   can significantly modify life history and reproduction traits in fish stocks, resulting in

496   individuals maturing earlier and at smaller sizes (Heino et al. 2015). The heritable component

497   of fishery effects on fish stocks is known 'fisheries-induced evolution' (see Heino et al.

498   2015); which has been recently examined from a physiological point of view (see Hollins et

499   al. 2018). In South Australia, octopus depredation occurs mainly on large male lobsters (size

500   and sex-dependant mortality), mirroring lobster catchability in this fishery (Briceño et al.

501   2015). It is unclear whether octopus depredation can also lead to an impact on lobster

502   populations by exacerbating the consumption of large individuals which are also targeted by

503   the lobster fishery. Likewise, reproductive females can also be targeted by octopus

504   depredation throughout the fishing season (Briceño et al. 2015), potentially impacting shifts

505   in lobster population traits. This deserves further attention of future studies examining lobster

506   physiological and behavioural responses under predation as only males were used in this

507   study.

508   Spiny lobsters moving to new areas, and therefore experiencing a change in habitat, is a well-

509   known response to predation risk (Childress & Jury, 2006). If J. edwardsii moves to new

510   habitats under predation risk, the type of refuge and level of food availability can be crucial

511   for survival, especially at early life history stages as juveniles are more dependent on habitat

512   conditions (Herrnkind et al. 1997). A reduction in sheltering areas can modify physiological

513   traits in prey individuals, as the lack of shelter can increase maintenance requirements in

514   aquatic organisms (Millidine et al. 2006; Toscano & Monaco 2015). Such physiological

515   alterations may be more elevated in habitats where both lobsters and octopus can compete for

516   shelters (e.g. *P. argus*, Berger & Butler 2001). Although in some rock lobster species (*P.*

517   *interruptus*), it is possible to find individuals sharing shelter space with octopus during day

518   time. This may lead to profound alterations in lobster physiology associated with the re-

519   allocation of resources from growth and reproduction to survival, resulting in altered

520   demographic traits as demonstrated in other taxa (Zanette et al. 2014) and which should be

521   examined by future studies on *J. edwardsii*.

522   Contrasting lobster life history, reproductive, behavioural and physiological traits with

523   different levels of predation risk by octopus could be addressed by future research to examine

524   the effect of fishing in shaping this predator-prey interaction. In doing so, future

525   examinations should also evaluate behavioural and physiological responses by lobsters under

526   a mortality threat in more complex trophic habitats such as marine protective areas, as lobster

527   are able to modify important behavioural defensive traits (e.g shelter dwelling and

528   gregariousness) in comparison with fishing areas (see Loflen & Hovel, 2010).

529   New disciplines integrating eco-physiological traits of aquatic organisms into management

530   and conservation decision-making are quickly emerging (i.e conservation physiology, Cooke

531   et al., 2013), and outcomes from the current study may serve as a base line for future studies

532   on eco-physiological traits and predator-prey interactions.

533

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548   7. References

549 Atema J (1995) Chemical signals in the marine environment: dispersal, detection, and temporal

550   signal analysis. P Natl Acad Sci USA 92:62–66

551 Berger DK, Butler MJ (2001) Octopuses influence den selection by juvenile Caribbean spiny

552   lobster. Mar Freshwater Res 52:1049–1053

553 Berriman JS, Kay MC, Reed DC, Rassweiler A, Goldstein DA, Wright WG (2015) Shifts in

554   attack behavior of an important kelp forest predator within marine reserves. Mar Ecol Prog

555   Ser 522: 193-201

556 Boal JG, Golden DK (1999) Distance chemoreception in the common cuttlefish, *Sepia officinalis*

557   (Mollusca, Cephalopoda). J Exp Mar Biol Ecol 235:307–317

558 Boyle PR (1983) Ventilation rate and arousal in the octopus. J Exp Mar Biol Ecol 69:129–136

559 Briceño F, Linnane AJ, Quiroz JC, Gardner C, Pecl GT (2015) Predation risk within fishing gear

560   and its implications for Australian southern rock lobster fisheries. PLOS ONE 10:e0139816

561 Briceño F, León R, Gardner C, Hobday, A, André J, Frusher S, Pecl GT (2016) Spatial variation

562   in mortality by within-pot predation in the Tasmanian rock lobster fishery. Fish Oceanogr 25:

563   6-18

564 Briones-Fourzán P (2009) Assessment of predation risk through conspecific alarm odors by

565   spiny lobsters: How much is too much? Commun Integr Biol 2:302–304

566 Brock D, Saunders T, Ward TM (2003) Development and assessment of methods to reduce

567   predation of "pot caught" southern rock lobster. Adelaide: SARDI Aquatic Sciences; Jun.

568   Report No.: RD03/0063

569 Brock D, Ward TM (2004) Maori octopus (*Octopus maorum*) bycatch and southern rock lobster

570   (*Jasus edwardsii*) mortality in the South Australian rock lobster fishery. Fish B-NOAA

571   102:430–440

572 Brooks WR (1991) Chemical recognition by hermit-crabs of their symbiotic sea-anemones and a

573   predatory octopus. Hydrobiologia 216:291–295

574 Bouwma PE, Herrnkind WF (2009) Sound production in Caribbean spiny lobster *Panulirus*

575   *argus* and its role in escape during predatory attack by *Octopus briareus*. New Zeal J Mar

576   Fresh 43:3–13

577 Brown C, Gardner C, Braithwaite VA (2005) Differential stress responses in fish from areas of

578   high- and low-predation pressure. J Comp Physiol B 175:305–312

579 Buscaino G, Filiciotto F, Gristina M, Buffa G, Bellante A, Maccarrone V, Patti B, Mazzola S

580   (2011) Defensive strategies of European spiny lobster *Palinurus elephas* during predator

581   attack. Mar Ecol Prog Ser 423:143–154

582 Butler MJ, MacDiarmid AB, Booth JD (1999) The cause and consequence of ontogenetic

583   changes in social aggregation in New Zealand spiny lobsters. Mar Ecol Prog Ser 188:179–

584   191

585 Butler MJ, Lear JA (2009) Habitat-based intraguild predation by caribbean reef octopus *Octopus*

586 *briareus* on juvenile caribbean spiny lobster *Panulirus argus*. Mar Ecol Prog Ser 386:115–

587 122

588 Careau V, Thomas D, Humphries MM, & Réale D (2008). Energy metabolism and animal

589 personality. Oikos, 117(5): 641–53

590 Clark TD, Sandblom E, Jutfelt F (2013). Aerobic scope measurements of fishes in an era of

591 climate change: respirometry, relevance and recommendations. J Exp Biol 216: 2771–2782

592 Cooke SJ, Steinmetz J, Degner JF, Grant EC, Philipp DP (2003) Metabolic fright responses of

593 different-sized largemouth bass (*Micropterus salmoides*) to two avian predators show

594 variations in nonlethal energetic costs. Can J Zool 81:699–709

595 Cooke SJ, Sack L, Franklin CE, Farrell AP, Beardall J, Wikelski M, Chown SL (2013) What is

596 conservation physiology? Perspectives on an increasingly integrated and essential science.

597 Conserv Physiol 1(1): 1-23

598 Cooke SJ, Killen SS, Metcalfe JD, McKenzie DJ, Mouillot D, Jørgensen C, Peck MA (2014)

599 Conservation physiology across scales: insights from the marine realm. Conserv Physiol 2:1–

600 15

601 Crear BJ, Forteath GNR (2000) The effect of extrinsic and intrinsic factors on oxygen

602 consumption by the southern rock lobster, *Jasus edwardsii*. J Exp Mar Biol Ecol 252:129–

603 147

604 Crear BJ, Forteath GNR (2001) Flowrate requirements for captive western rock lobsters

605 (*Panulirus cygnus*): effects of body weight, temperature, activity, emersion, daily rhythm,

606 feeding and oxygen tension on oxygen consumption. Mar Freshwater Res 52:763–771

607 Childress MJ, Herrnkind WF (1994) The behavior of juvenile Caribbean spiny lobster in Florida

608   Bay: seasonality, ontogeny and sociality. B Mar Sci 54:819–827

609 Childress MJ, Jury SH 2006. Behaviour. In: Phillips BF (ed) The biology of lobsters. Oxford,

610   United Kingdom and Ames, United States, Blackwell Publishing. pp. 78-112

611 Chivers DP, Dixson DL, White J, McCormick MI, Ferrari MOC (2013) Degradation of chemical

612   alarm cues and the assessment of risk throughout the day. Ecol Evol 3:3925–3934

613 Dall W (1986) Estimation of routine metabolic rate in a penaeid prawn, *Penaeus esculentus*

614   Haswell. J Exp Mar Biol Ecol 96:57–74

615 Díaz ER, Thiel M (2004) Chemical and visual communication during mate searching in rock

616   shrimp. Biol Bull 206:134–143

617 England WR, Baldwin J (1983) Anaerobic energy metabolism in the tail musculature of the

618   Australian yabby Cherax destructor: role of phosphagens and anaerobic glycolysis during

619   escape behavior. Physiol Zool 56:614–622

620 Ferrari MCO, Wisenden BD, Chivers DP (2010) Chemical ecology of predator–prey interactions

621   in aquatic ecosystems: a review and prospectus. Can J Zool 88:698–724

622 Fitzgibbon QP, Ruff N, Tracey SR, Battaglene SC (2014a) Thermal tolerance of the nektonic

623   puerulus stage of spiny lobsters and implications of ocean warming. Mar Ecol Prog Ser

624   515:173–186

625 Fitzgibbon QP, Jeffs AG, Battaglene SC (2014b) The Achilles heel for spiny lobsters: the

626   energetics of the non-feeding post-larval stage. Fish Fish 15:312-326

627 Gravel MA, Suski CD, Cooke SJ (2011) Behavioral and physiological consequences of nest

628   predation pressure for larval fish. Behav Ecol 22:510–519

629 Gristina M, Sinopoli M, Fiorentino F, Garofalo G, Badalamenti F (2011) Shelter selection of the

630 spiny lobster *Palinurus elephas* under different levels of *Octopus vulgaris* predation threat.

631 Mar Biol 158: 1331–1337

632 Groeneveld G, Maharaj C, Smith D (2006) *Octopus magnificus* predation and bycatch in the trap

633 fishery for spiny lobsters *Palinurus gilchristi* off South Africa. Fish Res 79:90–96

634 Hanlon RT, Messenger JB (1998) Cephalopod Behaviour. Cambridge University Press, London

635 Harrington JJ, Semmens JM, Gardner C, Frusher SD (2006) Predation of trap-caught southern

636 rock lobsters, *Jasus edwardsii* (Hutton, 1875), in Tasmanian waters by the Maori octopus,

637 *Octopus maorum* (Hutton, 1880): Spatial and temporal trends. Fish Res 77:10–16

638 Hawlena D, Schmitz OJ (2010) Physiological stress as a fundamental mechanism linking

639 predation to ecosystem functioning. Am Nat 176:537–556

640 Hazlett BA (2011) Chemical cues and reducing the risk of Predation. In: Breithaupt T, Thiel M

641 (eds) Chemical communication in crustaceans. Springer, New York, pp. 356–370

642 Helfman GS (1989) Threat-sensitive predator avoidance in damselfish-trumpetfish interactions.

643 Behav Ecol Sociobiol 24:47–47

644 Heino M, Díaz Pauli B, Dieckmann U (2015) Fisheries-induced evolution. Ann Rev Ecol Evol S

645 46: 461–480

646 Herrnkind WF, Butler MJ, Hunt JH, Childress M (1997) Role of physical refugia: implications

647 from a mass sponge die-off in a lobster nursery in Florida. Mar. Freshwater Res 48: 759–769

648 Herrnkind WF, Childress MJ, Lavalli KL (2001) Cooperative defence and other benefits among

649 exposed spiny lobsters: Inferences from group size and behaviour. Mar Freshwater Res

650 52:1113–1124

651 Herskin J (1999) Effects of social and visual contact on the oxygen consumption of juvenile sea

652   bass measured by computerized intermittent respirometry. J Fish Biol 55:1075–1085

653 Hollins J, Thambithurai D, Koeck B, Crespel A, Bailey DM, Cooke SJ, Lindström J, Parsons KJ,

654   Killen SS (2018) A physiological perspective on fisheries-induced evolution. Evol Appl

655   11(5): 561-576

656 Holopainen IJ, Aho J, Vornanen M, Huuskonen H (1997) Phenotypic plasticity and predator

657   effects on morphology and physiology of crucian carp in nature and in the laboratory. J Fish

658   Biol 50:781–798

659 Horner AJ, Nickles SP, Weissburg MJ, Derby CD 2006 Source and specificity of chemical cues

660   mediating shelter preference of Caribbean spiny lobsters (*Panulirus argus*). Biol Bull

661   211:128-139

662 Hunter CM, Haddon M, Sainsbury KJ (2005) Use of fishery-dependent data for the evaluation of

663   depensation: case study involving the predation of rock lobster (*Jasus edwardsii*) by octopus

664   (*Octopus maorum*). New Zeal J Mar Fresh 39:455-469

665 Jensen MA, Fitzgibbon QP, Carter CG, Adams LR (2013a) Effect of body mass and activity on

666   the metabolic rate and ammonia-N excretion of the spiny lobster *Sagmariasus verreauxi*

667   during ontogeny. Comp Biochem Phys A 166:191–198

668 Jensen MA, Fitzgibbon QP, Carter CG, Adams LR (2013b) Recovery periods of cultured spiny

669   lobster, *Sagmariasus verreauxi* juveniles: Effects of handling, force feeding, exercising to

670   exhaustion and anaesthesia on oxygen consumption and ammonia-N excretion rates.

671   Aquaculture 410–411:114–121

672 Jimenez AG, Locke BR, Kinsey ST (2008) The influence of oxygen and high-energy phosphate

673   diffusion on metabolic scaling in three species of tail-flipping crustaceans. J Exp Biol

674   211:3214–3225

675 Kats LB, Dill LM (1998) The scent of death: Chemosensory assessment of predation risk by prey

676   animals. Ecoscience 5:361–394

677 Kemp JOG, Britz PJ, Cockcroft AC (2009) Effect of body size, photophase, feeding and

678   emersion on the oxygen consumption of the east coast rock lobster *Panulirus homarus*

679   *rubellus*. Aquac Res 40:833–844

680 Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and

681   prospectus. Can J Zool 68:619-640

682 Lima SL, Bednekoff PA (1999) Temporal variation in danger drives antipredator behavior: the

683   predation risk allocation hypothesis. Am Nat 153:649–659

684 Lima SL, Steury T (2005) Perception of predation risk: the foundation of nonlethal predator-prey

685   interactions. In: Barbosa P, Castellanos I (eds) Ecology of predator-prey interactions. Oxford

686   University Press, New York, pp. 166–188

687 Loflen CL, Hovel KA (2010) Behavioral responses to variable predation risk in the California

688   spiny lobster *Panulirus interruptus* Mar Ecol Prog Ser 420:135–144

689 Lucas A (1993) Bioénergétique Des Animaux Aquatiques. Masson, Paris, pp. 179

690 MacDiarmid AB, Hickey B, Maller RA (1991) Daily movement patterns of the spiny lobster

691   *Jasus edwardsii* (Hutton) on a shallow reef in northern New Zealand. J Exp Mar Biol Ecol

692   147:185–205

693 Matassa CM, Trussell GC (2014) Prey state shapes the effects of temporal variation in predation

694   risk. P R Soc B 281:20141952

695 Millidine KJ, Armstrong JD, Metcalfe NB (2006). Presence of shelter reduces maintenance

696   metabolism of juvenile salmon. Funct Ecol 20(5): 839–845

697 Mills DJ, Verdouw G, Frusher SD (2005). Remote multi-camera system for in situ observations

698   of behaviour and predator/prey interactions of marine benthic macrofauna. New Zeal J Mar

699   Fresh 39: 347-352

700 Mills DJ, Johnson CR, Gardner C (2008) Bias in lobster tethering experiments conducted for

701   selecting low-predation release sites. Mar Ecol Prog Ser 364:1–13

702 Murdoch WW, Briggs CJ, Nisbet RM (2003) Consumer-resource dynamics. Princeton

703   University Press, Princeton.

704 Murdoch WW, Oaten A (1975) Predation and population stability. Adv Ecol Res 9:2–132

705 Nilsson DE, Warrant EJ, Johnsen S, Hanlon R, Shashar N (2012) A unique advantage for giant

706   eyes in giant squid. Curr Biol 22:683–688

707 Okuyama T (2015) Metabolic responses to predation risk in a jumping spider. J Zool 297:9-14

708 Phillips BF, Wahle RA, Ward T (2012) Lobsters as part of marine ecosystems – a review. In:

709   Phillips BF (ed) Lobsters: biology, management, aquaculture and fisheries. Wiley-Blackwell

710   Publishing, Oxford, pp. 1–30

711 Polese G, Bertapelle C, Di Cosmo A (2015) Role of olfaction in *Octopus vulgaris* reproduction.

712   Gen Comp Endocr 210:55–62

713 Preisser EL, Bolnick DI, Bernard MF (2005) Scared to Death? The effects of intimidation and

714   consumption in predator–prey interactions. Ecology 86:501–509

715 Ritchie L (1972) Octopus predation on trap-caught rock lobster—Hokianga area, N.Z. New

716   Zealand Marine Department, Fisheries Technical Report No.: 81

717 Ross D, Boletzky S (1979) The association between the pagurid *Dardanus arrosor* and the

718   actinian *Calliactis parasitica*. Recovery of activity in "inactive" D. arrosor in the presence of

719   cephalopods. Mar Behav Physiol 6:175–184

720 Sakamoto R, Ito A, Wada S (2006) Combined effect of risk type and activity rhythm on anti-

721   predator response of the shore crab *Gaetice depressus* (Crustacea: Grapsidae). J Mar Biol

722   Assoc UK 86:1401-1405

723 Shabani S, Kamio M, Derby CD (2008) Spiny lobsters detect conspecific blood-borne alarm cues

724   exclusively through olfactory sensilla. J Exp Biol 211:2600–2608

725 Shabani S, Kamio M, Derby CD (2009) Spiny lobsters use urine-borne olfactory signaling and

726   physical aggressive behaviors to influence social status of conspecifics. J Exp Biol 212: 2464

727   - 2474

728 Sheriff MJ, Thaler JS (2014) Ecophysiological effects of predation risk; an integration across

729   disciplines. Oecologia 176:607–611

730 Sih A (1985) Evolution, predator avoidance and unsuccessful predation. Am Nat 125:153–157

731 Sih A (1992) Prey uncertainty and the balancing of antipredator and feeding needs. Am Nat

732   139:1052–1069

733 Slos S, Stoks R (2008) Predation risk induces stress proteins and reduces antioxidant defense.

734   Funct Ecol 22:637–642

735 Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. WH Freeman and Co, New York, pp. 1-887.

736 Steiner UK, Van Buskirk J (2009) Predator-induced changes in metabolism cannot explain the

737   growth/predation risk tradeoff. PLOS ONE 4:2–5

738 Toscano BJ, Monaco CJ (2015) Testing for relationships between individual crab behavior and

739   metabolic rate across ecological contexts. Behav Ecol Sociobiol 69 (8):1-9

740 Trussell GC, Ewanchuk P, Matassa CM (2006) The fear of being eaten reduces energy transfer in

741   a simple food chain. Ecology 87:2979–2984

742 Trussell GC, Schmitz OJ (2012) Species functional traits trophic control and the ecosystem

743   consequences of adaptative foraging in the middle of food chains. In: Ohgushi T, Schmitz O,

744   Holt R (eds) Trait-Mediated Indirect Interactions: Ecological and Evolutionary Perspectives.

745   Cambridge University Press, Cambridge, pp. 296–324

746 Uhlmann SS, Broadhurst MK (2013) Mitigating unaccounted fishing mortality from gillnets and

747   traps. Fish and Fisheries 16 (2): 183-229

748 Weiss HM, Lozano-Álvarez E, Briones-Fourzán P, Negrete-Soto F (2006) using red light with

749   fixed-site video cameras to study the behavior of the spiny lobster, *Panulirus argus*, and

750   associated animals at night and inside their shelters. Mar Technol Soc J 40:86–95

751 Weiss HM, Lozano-Álvarez E, Briones-Fourzán P (2008) Circadian shelter occupancy patterns

752   and predator-prey interactions of juvenile Caribbean spiny lobsters in a reef lagoon. Mar Biol

753   153: 953–963

754 Werner EE, Peacor SD (2003) A review of trait- mediated indirect interactions in ecological

755   communities. Ecology 84:1083–1100

756 Williams BG, Dean IC (1989) Timing of locomotor activity in the New Zealand rock lobster,

757   *Jasus edwardsii*. New Zeal J Mar Fresh 23:215–224

758 Withy-Allen KR, Hovel KA (2013) California spiny lobster (*Panulirus interruptus*) movement

759   behaviour and habitat use: implications for the effectiveness of marine protected areas. Mar

760   Fresh Res 64: 359–371

761 Zanette LY, Clinchy M, Suraci JP (2014) Diagnosing predation risk effects on demography: can

762   measuring physiology provide the means? Oecologia 176:637–651

763 Ziegler P, Haddon M, Frusher S, Johnson C (2004) Modelling seasonal catchability of the

764   southern rock lobster *Jasus edwardsii* by water temperature, moulting, and mating. Mar Biol

765   145:179–190

766 Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GA (2009) Mixed Effects Models and

767   Extensions in Ecology with R. Springer-Verlag, New York, pp. 1-574.

768

770
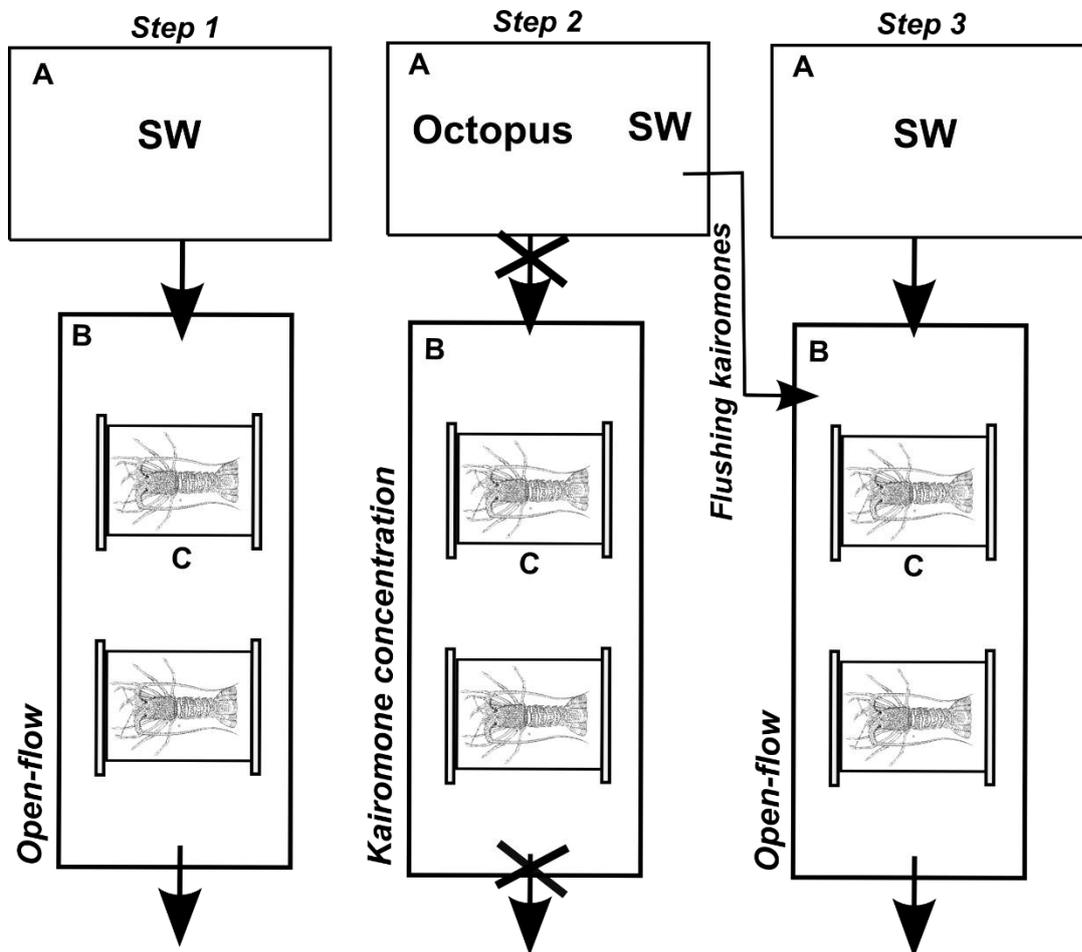
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772 Fig. 1: Protocol to depict nocturnal predation risk scenarios used in *Jasus edwardsii* adult

773 respirometry. The experimental set up consisted of three units: the conditioning tank (A), the

774 treatment tank or 'bath' (B) and the respirometric chambers (C). The units A and B were

775 connected by an open-flow circulation of sea water (SW) indicated with black arrows. The

776 protocol used to depict the predation risk scenario is as follows: (Step 1) Two lobsters were

777 acclimated for six hours in the unit C with an open-flow circulation. (Step 2) An octopus was

778 held in A for one hour and the open-flow was stopped over this period. (Step 3) The octopus

779 was removed and kairomones was flushed into B.
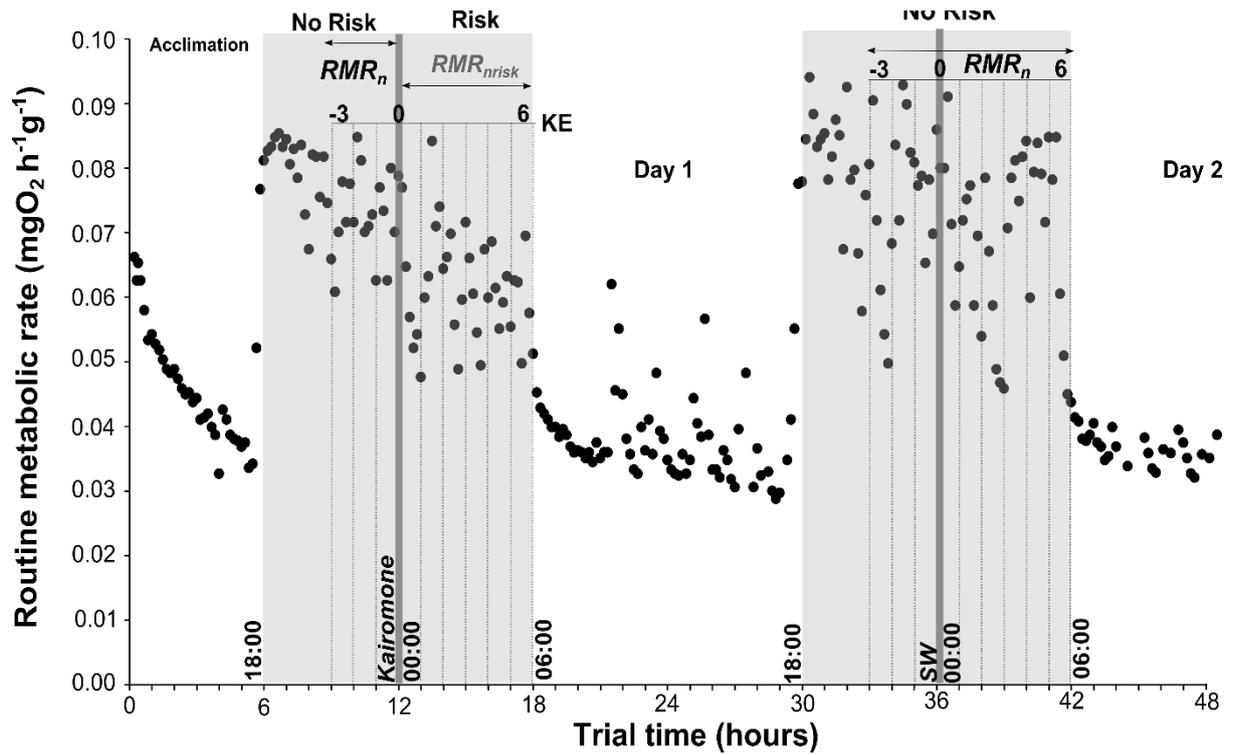
Fig. 2: Example of a respirometry trial for *Jasus edwardsii* adults (700 g; 17°C) for 48 hours
to describe the nocturnal predation risk scenario. Lobsters were acclimated for six hours into
the respirometric chambers. Night time is specified by light grey boxes (18:00 and 06:00).
Each dot represents one measurement of metabolic rate (mg O2 h^{-1} g^{-1}) taken every 10
minutes. Routine metabolic rate (RMR) was calculated as hourly mean resulting in six
measurements per hour. Nocturnal RMR (RMR_n) was examined over two consecutive nights
(night 1 and night 2) where one of the two treatments (absence/presence of kairomones) was
applied (approximately at midnight). In this example, lobster was exposed to octopus cues the
first night and the resulting rates after kairomone exposure (KE) (vertical solid lines) were
specified as RMR_{nrisk}. In the second night the same protocol was applied but only including
sea water (SW) with the resulting rate used as control and referred as RMR_n.
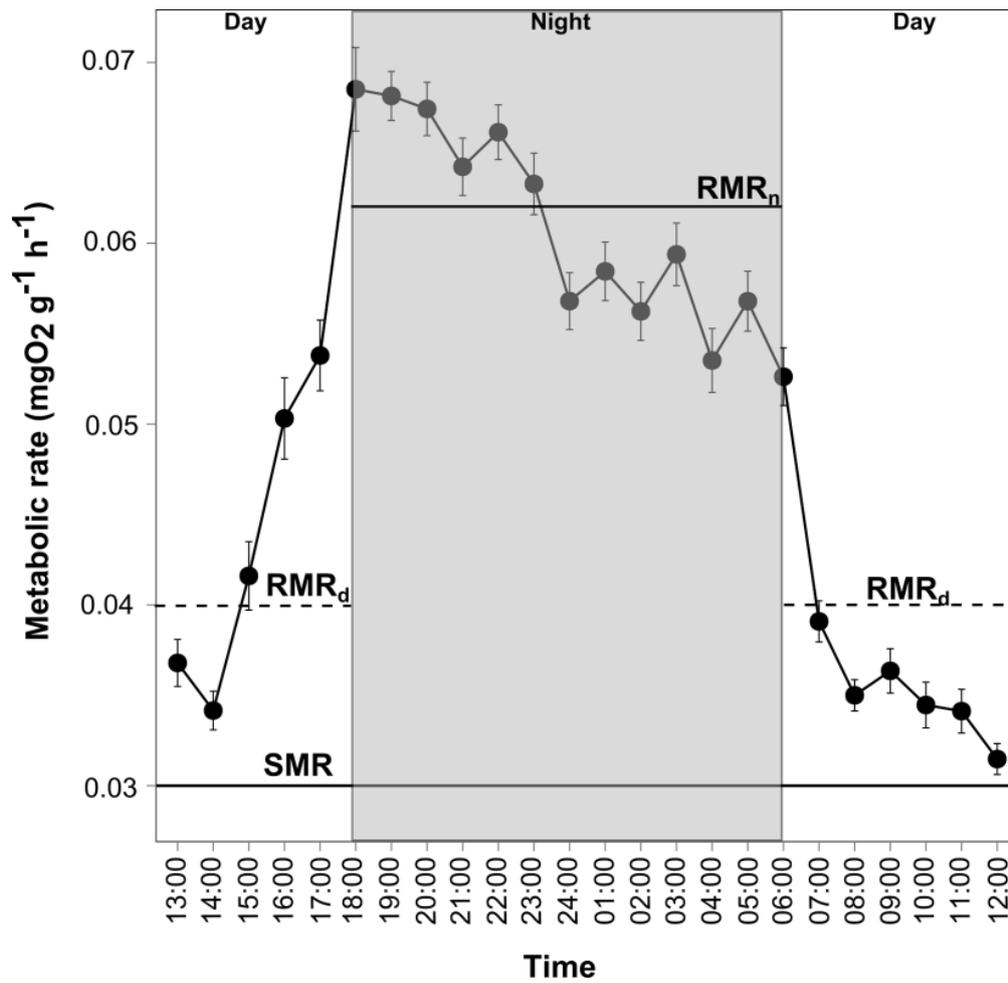
Fig. 3: Circadian pattern in mean $\pm$ (SE) routine metabolic rate (RMR) in adults *Jasus edwardsii* (n = 16, 514–732 g, 17 °C). The grey box represents the nocturnal period between 18:00 and 06:00. Mean values of RMR during night time (RMRn = 0.062 mg O_2 h^{-1} g^{-1}) and day time (RMRd = 0.040 mg O_2 h^{-1} g^{-1}) are specified by the upper (solid) and dashed lines respectively. Mean value of standard metabolic rate (SMR = 0.03 mg O_2 h^{-1} g^{-1}) is specified as a lower solid line.
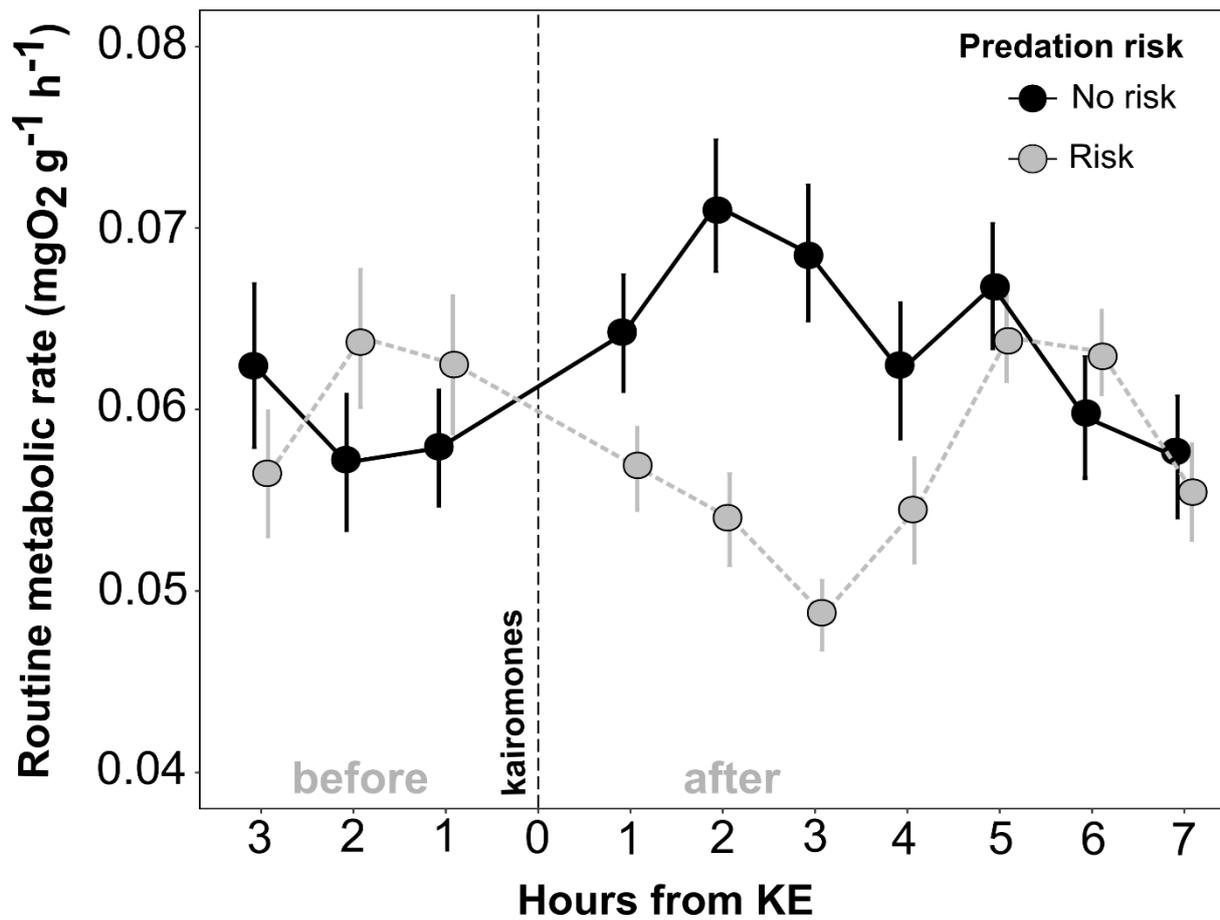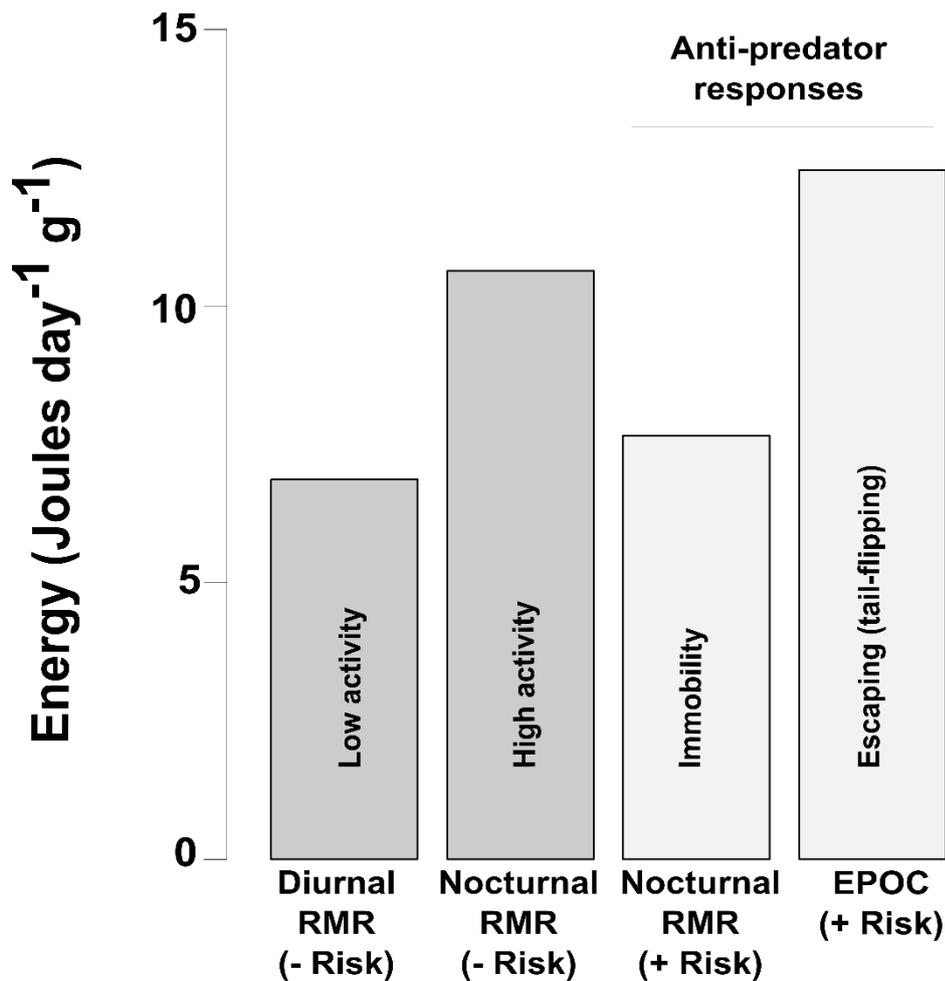
Fig. 4: Mean ± (SE) Routine metabolic rate of *Jasus edwardsii* adult under nocturnal predation risk scenarios (n = 10; 461–769 g). The vertical dashed line specifies the time when kairomones (octopus odour) were added (approximately at midnight), here referred as hours after kairomone exposure (KE).

Fig. 5: Daily energy requirements for lobster activity using routine metabolic rate (RMR) at two levels from circadian pattern (dark grey bars): low activity (diurnal RMR) and high activity (nocturnal RMR) without predation risk (- Risk) which are compared with two anti-predator responses (light grey bars): immobility from the lowering in nocturnal RMR under predation risk (+ Risk) here reported and escaping or tail-flipping from Jensen et al. (2013b). Diurnal and nocturnal RMR were calculated assuming 12 h day – 12 h night period. RMR under risk was calculated as 31.4 % of nocturnal RMR (- risk), assuming to be constant over nocturnal period (12 h). The escaping response, in addition, was calculated using the excess-post exercise oxygen consumption (EPOC) from Jensen et al. (2013b), representing the

819   energy required for a single escaping event performing tail-flipping until exhaustion (see

820   Jensen et al. 2013b).

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825   APPENDIX

826   Table A1: Behavioural studies using octopus – crustacean interactions as predator–prey

827   models, indicating field (F) or laboratory (L) investigations.

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Prey	Cue type	Prey response	Reference
Shore crab (*Gaetice depressu*)	Chemical	Reduction of activity	Sakamoto et al. 2006 (L)
European spiny lobster (*Palinurus elephas*)	Visual, chemical and tactile	Avoidance	Gristina et al. 2011 (L)
European spiny lobster (*Palinurus elephas*)	Visual, chemical and tactile	Avoidance	Buscaino et al. 2011 (L)
Caribbean spiny lobster (*Panulirus argus*)	Chemical	Avoidance	Berger & Butler 2001 (F); Horner et al. 2006 (L); Butler & Lear 2009 (F)
Caribbean spiny lobster (*Panulirus argus*)	Visual and chemical	Shelter occupancy	Weiss et al. 2008

Hermit crabs (*Dardanus venosus* and *Pagurus pollicaris*)	Chemical	Avoidance	Ross & Boletzky 1979 (L) Brooks 1991 (L)

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