Resistance to starvation of first-stage juveniles of the Caribbean spiny lobster

Alí Espinosa-Magaña [Corresp., 1, 2] , **Enrique Lozano-Álvarez** [2] , **Patricia Briones-Fourzán** [2]

[1] Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Ciudad de México, México

[2] Unidad Académica de Sistemas Arrecifales, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Puerto Morelos, Quintana Roo, México

Corresponding Author: Alí Espinosa-Magaña
Email address: disarm22@hotmail.com

The non-feeding postlarva (puerulus) of spiny lobsters actively swims from the open ocean to the coastal habitats where it settles and molts to the first-stage juvenile (JI). Because pueruli use much of their energy reserves swimming and preparing for the post-settlement molt, the survival of JIs presumably depends on resuming feeding as soon as possible. To test this hypothesis, the resistance to starvation of JIs of the Caribbean spiny lobster, *Panulirus argus*, was evaluated by measuring their point-of-no-return (PNR, minimum time of initial starvation preventing recovery after later feeding) and point-of-reserve-saturation (PRS, minimum time of initial feeding allowing for food-independent development through the rest of the molting cycle) in a warm and a cold season. Each experiment consisted of continuously fed and starved control groups (FC and SC, respectively) and six groups subjected to differential periods of initial starvation and subsequent feeding (PNR experiments) or initial feeding and subsequent starvation (PSR experiments). No JIs molted under continuous absence of food (SC). In both PNR experiments (temperature in warm season: 29.79 ± 0.07°C, mean ± 95% CI; in cold season: 25.63 ± 0.12°C) mortality increased sharply after 9 d of initial starvation and intermolt periods increased with period of initial starvation, but were longer in the cold season. The PNR_{50} was longer in the warm season (12.1 ± 1.2 d, mean ± 95% CI) than in the cold season (9.5 ± 2.1 d). In PRS experiments (temperature in warm season: 29.54 ± 0.07 °C; in cold season: 26.20 ± 0.12 °C), JIs that molted did so near the end of the feeding period; all JIs initially fed for up to 6 d succumbed, and no JIs molted after 13 d of starvation despite having fed previously. The PRS_{50} did not differ between the cold (13.1 ± 0.7 d) and warm seasons (12.1 ± 1.1 d). JIs of *P. argus* exhibit a remarkable resistance to starvation considering that the previous non-feeding, energy-demanding puerulus phase lasts for ~3 weeks. However, JIs appear to have a relatively higher degree of dependence on food to complete development to JII during the cold season than during the warm season. Therefore, JIs of *P. argus* would appear to be more resistant to starvation during the warm season.

1 **Resistance to starvation of first-stage juveniles of the Caribbean spiny lobster**

3 Alí Espinosa-Magaña [a,b,*], Enrique Lozano-Álvarez [b], and Patricia Briones-Fourzán [b]

5 [a] Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México,

6 Ciudad Universitaria, Ciudad de México, México.

8 [b] Unidad Académica de Sistemas Arrecifales, Instituto de Ciencias del Mar y Limnología,

9 Universidad Nacional Autónoma de México, Puerto Morelos, Quintana Roo, México.

11 Corresponding author: Alí Espinosa-Magaña

12 Email: disarm22@hotmail.com

25 **ABSTRACT**

26 The non-feeding postlarva (puerulus) of spiny lobsters actively swims from the open ocean to the

27 coastal habitats where it settles and molts to the first-stage juvenile (JI). Because pueruli use

28 much of their energy reserves swimming and preparing for the post-settlement molt, the survival

29 of JIs presumably depends on resuming feeding as soon as possible. To test this hypothesis, the

30 resistance to starvation of JIs of the Caribbean spiny lobster, *Panulirus argus*, was evaluated by

31 measuring their point-of-no-return (PNR, minimum time of initial starvation preventing recovery

32 after later feeding) and point-of-reserve-saturation (PRS, minimum time of initial feeding

33 allowing for food-independent development through the rest of the molting cycle) in a warm and

34 a cold season. Each experiment consisted of continuously fed and starved control groups (FC and

35 SC, respectively) and six groups subjected to differential periods of initial starvation and

36 subsequent feeding (PNR experiments) or initial feeding and subsequent starvation (PSR

37 experiments). No JIs molted under continuous absence of food (SC). In both PNR experiments

38 (temperature in warm season: $29.79 \pm 0.07°C$, mean $\pm$ 95% CI; in cold season: $25.63 \pm 0.12°C$)

39 mortality increased sharply after 9 d of initial starvation and intermolt periods increased with

40 period of initial starvation, but were longer in the cold season. The PNR_{50} was longer in the

41 warm season (12.1 ± 1.2 d, mean $\pm$ 95% CI) than in the cold season (9.5 ± 2.1 d). In PRS

42 experiments (temperature in warm season: $29.54 \pm 0.07 °C$; in cold season: $26.20 \pm 0.12 °C$), JIs

43 that molted did so near the end of the feeding period; all JIs initially fed for up to 6 d succumbed,

44 and no JIs molted after 13 d of starvation despite having fed previously. The PRS_{50} did not differ

45 between the cold (13.1 ± 0.7 d) and warm seasons (12.1 ± 1.1 d). JIs of *P. argus* exhibit a

46 remarkable resistance to starvation considering that the previous non-feeding, energy-demanding

47 puerulus phase lasts for ~3 weeks. However, JIs appear to have a relatively higher degree of

48   dependence on food to complete development to JII during the cold season than during the warm

49   season. Therefore, JIs of *P. argus* would appear to be more resistant to starvation during the

50   warm season.

51

52   **INTRODUCTION**

53   The Caribbean spiny lobster, *Panulirus argus* (Latreille, 1804) is widely distributed in the

54   wider Caribbean region (Holthuis, 1991), where it constitutes one of the most important fishing

55   resource (Phillips et al., 2013). However, in various parts of the Caribbean, including Mexico,

56   catches of *P. argus* have suffered a substantial decline since the late 1990s (Aguilar et al., 2003;

57   Ehrhardt, Puga & Butler, 2011).

58   In the warm Caribbean waters, *P. argus* breeds throughout most of the year, with a major

59   peak during the spring and a secondary peak in the fall (Briones et al., 1997). The larval phase

60   consists of 10 flattened planktonic stages, the phyllosomata (Goldstein et al., 2008). Larval

61   development takes place in oceanic waters and lasts 5-9 months, conferring an enormous

62   dispersion potential. The final larval stage undergoes a drastic metamorphosis into the postlarval

63   phase, which consists of a single nektonic stage, the puerulus, which is more similar in shape to

64   an adult lobster but is completely transparent (Phillips et al., 2006). Unlike phyllosomata, which

65   are planktotrophic, pueruli do not feed, i.e., they represent a secondary lecithotrophic phase that

66   depends on its own energy reserves (Kittaka, 1994; Lemmens, 1994; McWilliam & Phillips,

67   1997; Cox, Jeffs & Davies, 2008). Pueruli actively swim towards the shore and settle in shallow

68   coastal vegetated habitats, such as seagrass and macroalgal beds (Butler & Herrnkind, 2000;

69   Briones-Fourzán & Lozano-Álvarez, 2001). A few days after settling, pueruli molt into the first

70   juvenile stage, also known as post-puerulus (e.g. Limbourn et al., 2008), which resumes feeding.

71   Under laboratory conditions, the total duration of the non-feeding puerulus phase of *P.*

72   *argus* has been 16 to 26 d at 25° C and 11 to 18 d at 27° C (Goldstein et al., 2008). During this

73   period most of the puerulus energy is allocated to swimming and preparing for the post-

74   settlement molt into the first juvenile stage (JI), which involves further morphological changes

75   (Lewis, Moore & Babis, 1952; Goldstein et al., 2008; Ventura et al., 2015). Swimming for

76   distances of up to tens of kilometers to the settlement site can severely deplete the energy stores

77   of the puerulus, potentially compromising its ability to molt into the JI (Jeffs, Willmott & Wells,

78   1999; Jeffs, Chiswell & Booth, 2001). Moreover, resumption of feeding by the JI may depend

79   on local food availability and predation risk (Lozano-Álvarez, 1996; Weiss, Lozano-Álvarez &

80   Briones-Fourzán, 2008). If the JI cannot restore sufficient energy reserves quickly enough, it

81   could starve to death (Fitzgibbon, Jeffs & Battaglene, 2014a). Therefore, it is important to

82   determine the resistance to starvation (sensu Sulkin, 1978) of JIs of *P. argus*. This information,

83   in addition to increasing knowledge regarding the biology of the early benthic stages of this

84   species, is important for its potential in aquaculture and for the generation of predictive models

85   of local lobster production based on levels of puerulus settlement (Phillips et al., 2000).

86   In the present study, resistance to starvation of JIs of *P. argus* was experimentally

87   determined via two physiological indices, the point-of-no-return (PNR) and the point-of-reserve-

88   saturation (PRS). The PNR is the duration of initial food deprivation that will cause irreversible

89   damage, i.e., that will not allow recovery even after later re-feeding (Blaxter & Hempel, 1963;

90   Anger & Dawirs, 1981); the PRS is the minimum time of initial feeding after which a later food-

91   independent development to the next stage is possible (Anger, 1987, 1995). To our knowledge,

92   the PRS has not been determined for JIs of any spiny lobster species, and the only one for which

93   the PNR of JIs has been determined is *P. cygnus* (Limbourn et al., 2008). In the present study,

94   the PNR was determined by subjecting JIs of *P. argus* to a range of initial starvation periods

95   shortly after molting, followed by continuous feeding. The PRS was determined by feeding JIs

96   initially for diverse periods before food was permanently withheld. Each index was estimated

97   during a warm season (summer-autumn) and a cold season (winter-spring) because the metabolic

98   and growth rates of crustaceans in general (e.g. Anger, 2001), and of *P. argus* in particular

99   (Perera et al., 2007), increase with temperature, potentially affecting the PNR and PRS (Anger et

100   al., 1981; Paschke et al., 2004; Bas, Spivak & Anger, 2008; Gebauer, Pashke & Anger, 2010).

101

102   **MATERIALS AND METHODS**

103   Given the long larval duration and extremely high rates of mortality of phyllosomata

104   under laboratory conditions (Goldstein et al., 2008), our study was conducted using wild

105   postlarvae as in Limbourn et al. (2008). Pueruli of *P. argus* were obtained from a set of 12

106   artificial seaweed GuSi collectors (Gutiérrez-Carbonell, Simonín-Díaz & Briones-Fourzán,

107   1992) permanently deployed in the reef lagoons of Puerto Morelos (20°52′07″ N, 86°52′04″ W)

108   and Bahía de la Ascensión (19°49′50″ N, 87°27′09″ W), Mexico. These collectors are only

109   checked once a month because they are used for long-term monitoring of monthly pueruli

110   settlement. Although monthly settlement in collectors can vary by an order of magnitude within

111   a year, the average catch is 16 individuals per collector per month in Puerto Morelos, and 4

112   individuals per collector per month in Bahía de la Ascensión (Briones-Fourzán, Candela &

113   Lozano-Álvarez, 2008). Therefore, to increase the number of pueruli, a large mat (1 m × 8 m) of

114   the same artificial seaweed was moored off a dock at Puerto Morelos and checked for pueruli

115   every morning throughout the dark phase of the moon, when settlement levels are typically

116   higher (Briones-Fourzán, 1994). Within 1 h of collection, the pueruli were transported in aerated

117   seawater to the laboratory where the experiments were conducted. The necessary permits for

118   pueruli collection were obtained from Comisión Nacional de Acuacultura y Pesca

119   (DGOPA.06695.190612.1737).

120   The pueruli were individually placed in small covered cylindrical plastic baskets (0.5 l) to

121   allow free water exchange. Each basket was lined with black nylon mesh to prevent escape of the

122   puerulus and to reduce the light level. The baskets were partially submerged and supported by

123   ethylene vinyl acetate ("foamy") floats to provide buoyancy, and were distributed among three

124   tanks (2 m in diameter) with a water level of 0.7 m (approximately 2,800 l each), with 6–7

125   baskets allotted to each treatment in each tank. The tanks received seawater from an open-flow

126   system. The inflow water was pumped from the Puerto Morelos reef lagoon and passed through

127   an ozone chamber to a reservoir tank (6,300 l), then pumped through a sand filter (Hayward high

128   rate sand filter, S-244T) to remove suspended particles before its distribution to the experimental

129   tanks. The outflowing water was passed again through an ozone chamber. Water temperature in

130   the tanks was recorded twice a day with an YSI Environmental 556 multi-probe system.

131   Because each experiment required 160 pueruli (see below) but the number of pueruli that

132   could be collected during any given season (warm or cold) was limited, it was not possible to

133   conduct more than one experiment per season. Therefore, the PNR experiments were conducted

134   in June–October 2012 (warm season) and January–April 2013 (cold season), and the PRS

135   experiments were conducted in January–April 2014 (cold season) and July–November 2014

136   (warm season). For each experiment, 160 pueruli were divided into eight groups of 20

137   individuals each. All individuals were checked twice daily, in the morning and evening. When a

138   puerulus molted into a JI, the exuvia was removed. One group of recently molted JIs was

139   continuously fed (fed control, FC) until all individuals molted into second-stage juveniles (JIIs).

140   Another group remained unfed until all the individuals succumbed (starved control, SC). In the

141   PNR experiments, the six remaining groups of recently molted JIs were subjected to different

142   treatments (periods of starvation in multiples of 3 d) and then fed continuously. Thus, one group

143   was starved for 3 d and then fed (treatment S3), another group was starved for 6 d and then fed

144   (treatment S6), etc. (up to 18 d of initial starvation) (Fig. 1). In the PRS experiment, the six

145   remaining groups of recently molted JIs were also subjected to different treatments, consisting of

146   feeding periods (also in multiples of 3 d) and then starved. Thus, one group was fed for 3 d and

147   then starved (treatment F3), another group was fed for 6 d and then starved (treatment F6), etc.

148   (up to 18 d of initial feeding) (Fig. 1). Food, when provided, consisted of a piece of mussel meat

149   (*Perna canaliculus*) changed daily. Mussels are a good food source for early juveniles of

150   *Panulirus* in laboratory conditions, at least during the first four weeks after the molt to JI (e.g., *P.*

151   *cygnus*: Glencross et al., 2001; *P. ornatus*: Smith, Williams & Irvin, 2005; *P. interruptus*: Díaz-

152   Iglesias et al., 2011).Three-day periods were used to optimize the use of experimental individuals

153   (which are difficult to obtain in large numbers) given that, in the laboratory, the average

154   intermolt period of well-fed JIs of *P. argus* varied between 17 and 31 d depending on water

155   temperature (Lellis & Russell, 1990), whereas the maximal starvation time for JIs of *P. cygnus*

156   from which recovery was observed was 22 d (Limbourn et al., 2008). Therefore, JIs of *P. argus*

157   were expected to endure relatively long starvation periods.

158   For each individual, the first experimental day was one day after molting to JI (Limbourn

159   et al., 2008). The influence of the initial starvation periods (in the PNR experiments) or the initial

160   feeding periods (in the PRS experiments) was measured as percent mortality and the average

161   duration of the JI stage of individuals that molted to JII (intermolt period). For the PNR and PRS

162   experiments, duration of stage JI in days (logarithmically transformed to increase homogeneity

163   of variance) was separately subjected to a factorial ANOVA with season and treatment as fixed

164   factors. Starvation tolerance was quantified as the median point-of-no-return (PNR_{50}) and point-

165   of-reserve-saturation (PRS_{50}). PNR_{50} is the time when 50% of initially starved JIs lost the

166   capability to recover, even after subsequent feeding, and died without molting to JII; PRS_{50} is the

167   time when 50% of initially fed JIs attained the capability to develop through the rest of the

168   molting cycle using internally stored energy reserves (Anger & Dawirs, 1981). PNR_{50} and PRS_{50}

169   were estimated by fitting sigmoidal dose-response curves of cumulative mortality to the time of

170   initial starvation or feeding, respectively (Paschke et al., 2004; Gutow et al., 2007; Bas, Spivak

171   & Anger, 2008). Each index was compared between seasons (warm versus cold).

172

173   **RESULTS**

174   Throughout the text, results are reported as mean ± 95% CI unless otherwise stated.

175   Statistical results were considered as significant at $p < 0.05$.

176

177   **Point-of-no-return (PNR)**

178   In the PNR experiments, water temperature differed significantly between the warm

179   (29.79 ± 0.07 °C) and cold seasons (25.63 ± 0.12 °C) ($t_{176} = 28.65$, $p < 0.001$). The duration of

180   stage JI in continuously fed individuals (i.e., the FC) was longer in the cold season (24.5 ± 3.6 d,

181    $n = 17$) than in the warm season (18.2 ± 1.3 d, $n = 20$) ($t_{35} = 3.44$, $p < 0.001$). In general, the

182   duration of stage JI was significantly affected by season ($F_{1,148} = 68.04$, $p < 0.001$) and treatment

183   ($F_{5,148} = 22.32$, $p < 0.001$) but not by the interaction term ($F_{5,148} = 0.37$, $p = 0.87$); i.e., it

184   increased with the duration of the initial period of starvation in both seasons but was longer for

185   each period in the cold season (Fig. 2). Overall, mortality of JIs increased with number of initial

186   days of starvation, but tended to be higher in a few treatments of the cold season experiment (FC,

187   S3, S6, S12) than in the same treatments of the warm season experiment. The PNR_{50} was $12.1 \pm$

188   1.2 d in the warm season and 9.5 ± 2.1 d in the cold season (Fig. 3), a significant difference ($F_{1,}$

189    $_{12} = 7.924$, $p = 0.015$).

190

191   **Point-of-reserve-saturation (PRS_{50})**

192   In the PRS experiments, water temperature differed significantly between the cold season

193   (26.20 ± 0.12 °C) and the warm season (29.54 ± 0.07 °C) ($t_{285} = 24.23$, $p < 0.001$). The duration

194   of stage JI in individuals from the FC was significantly longer in the cold season (25.7 ± 2.3 d, n

195    $= 20$) than in the warm season (16.3 ± 1.3 d, $n = 20$) ($t_{38} = 7.05$, $p < 0.001$). Overall, the duration

196   of stage JI was significantly affected by season ($F_{1,119} = 37.9$, $p < 0.001$) and treatment ($F_{4,119} =$

197   8.71, $p < 0.001$), but the interaction term was also significant ($F_{4,119} = 3.76$, $p < 0.001$),

198   indicating that the intermolt period in the different treatments did not follow the same pattern in

199   both seasons (Fig. 4). In particular, the intermolt period was significantly longer in treatments

200   F15 and F18 and in the FC of the cold season than in the same treatments of the warm season

201   (Fig. 4).

202   Mortality of JIs decreased with increasing period of initial feeding, but was higher in

203   almost all treatments of the cold season than in those of the warm season. In both seasons, all

204   individuals in the SC and in treatments F3 and F6 succumbed. In both experiments, with the

205   exception of the fed control group, most JIs molted near the end of the initial feeding period,

206   with a maximum of 13 d between the end of the initial feeding period and molting. The PRS_{50}

207   was 13.1 ± 0.7 d in the cold season and 12.1 ± 1.1 d in the warm season (Fig. 5). These values

208   did not differ significantly ($F_{1, 12} = 3.603$, $p = 0.082$).

209

210 **DISCUSSION**

211   Temperature has an important effect on the physiology and ecology of ectotherms such as

212 spiny lobsters. For example, in Florida, USA, Witham (1973) reported that small juveniles of

213 *Panulirus argus* were intolerant of sustained temperatures below 15.6°C or above 30.0°C, and

214 that lobsters held below 20°C exhibited relatively little growth. In laboratory experiments held in

215 Cuba, oxygen consumption and metabolism of early benthic and older juveniles of *P. argus*

216 increased with temperature within the range of 19 to 30°C (Brito et al., 1991; Perera et al., 2007).

217 Lellis & Russell (1990) examined growth and survival of postpueruli and early juveniles of *P.*

218 *argus* at different temperatures and found that these stages grew faster at 30°C due to shorter

219 intermolt periods and greater size increments than at 24, 27, or 33°C. In our study, mean water

220 temperature during the PNR and PRS experiments was 25.9 and 26.2°C in the cold season,

221 respectively, and 29.8 and 29.5°C in the warm season, respectively. Consequently, in both types

222 of experiment the duration of stage JI of continuously fed individuals (FC) was significantly

223 longer in the cold season than in the warm season.

224   Limbourn et al. (2008) estimated the PNR_{50} of JIs of *P. cygnus*, a subtropical species, at

225 22 d, which is about twice the PNR_{50} estimated for the tropical *P. argus* in the present study.

226 Regardless, the resistance to starvation of JIs of *P. argus* is remarkable, considering the energetic

227 demand imposed on the non-feeding pueruli during their transit from oceanic waters to the coast

228 (e.g. Fitzgibbon, Jeffs & Battaglene, 2014a). As previously found by Limbourn et al. (2008) for

229 *P. cygnus*, no JIs of *P. argus* were able to molt in complete absence of food (SC). Dependence

230 on exogenous food of JIs to complete development has also been found in other decapods, even

231 in some species with facultative lecithotrophic postlarvae (Calado et al., 2010). An exception is

232   the red cherry shrimp, *Neocaridina davidi*, in which JIs were able to molt even in complete

233   absence of food, probably because this species has a much abbreviated post-hatching

234   development (Pantaleão et al., 2015). In our two PNR experiments, the duration of stage JI

235   increased with increasing period of initial starvation. A delay in molting caused by lack of food

236   has been documented in early life stages of other marine and freshwater crustacean species (e.g.,

237   Anger & Dawirs, 1981; Mikami, Greenwood & Gillespie, 1995; Abrunhosa & Kittaka, 1997;

238   Stumpf et al., 2010; Calvo et al., 2012) except *N. davidi,* in which the duration of stages JI and

239   JIII was not affected by the duration of initial starvation periods (Pantaleão et al., 2015).

240   In the PRS experiments, the few individuals that molted to JII in treatments F9 and F12

241   did so near the end of the feeding period, and no individuals molted after 13 d of starvation

242   despite having been previously fed, resulting in an apparent increase in duration of stage JI with

243   duration of initial feeding period. This result contrasts with the results of the PNR experiments,

244   in which seven individuals from treatments S15 and S18 of the warm season experiment and

245   eight from treatments S15 and S18 of the cold season experiment were able to molt. The higher

246   tolerance to starvation of individuals from the PNR experiments may be due to a suspension of

247   the molting cycle, i.e., an arrest of development in the intermolt stage (stage C in Drach's molt

248   classification system) of individuals subjected to initial starvation, with a subsequent shift to

249   premolt stage (stage D) with further feeding (Anger, 1987). However, in PRS experiments, if

250   individuals reach the premolt stage during the period of initial feeding and then are subjected to

251   continuous starvation, an unsuccessful attempt to molt will lead to death because an arrest of

252   development in premolt is not possible (Anger, 2001).

253   The PRS occurs during the transition between late intermolt (stage C_4) and early premolt

254   (stage D_0), which is also known as the "D_0 threshold", a critical period for crustaceans in terms

255   of nutritional requirements (Anger, 1987, 2001). The D_0 threshold has been confirmed in early

256   life stages of decapod crustaceans from different families, e.g., Crangonidae (Paschke et al.,

257   2004), Diogenidae (Harms, 1992), Majidae (Figueiredo et al., 2008; Guerao et al., 2012), and

258   Portunidae (Harms et al., 1990). In our study, results of both PRS experiments, with high

259   mortality rates in individuals subjected to treatments F3, F6 and F9, and shorter intermolt periods

260   and higher mortality rates than in PNR experiments, as well as the similarity in PRS_{50} values

261   between seasons (unlike in PNR_{50} values), suggest that feeding in *P. argus* JIs is more important

262   near the PRS (or D_0 threshold) than immediately after molting to the first-stage juvenile.

263   Although PRS_{50} values did not differ with season, the ratio of the PRS to the total

264   duration of stage JI in individuals from the FC was higher during the warm season (74%) than in

265   the cold season (51%). Therefore, despite the significantly longer duration of stage JI in the cold

266   season, the PRS was reached earlier than in the warm season. A similar pattern was observed in

267   the zoea I of the shrimp *Crangon crangon*, in which the ratio of the PRS to the stage duration

268   was 32% in the summer and 23% in the winter (Paschke et al., 2004). However, the PRS

269   represents about one-third of the total intermolt time in the early phases of many crustaceans,

270   including *C. crangon*, the zoeae I and II of the spider crab *Hyas araneus* (Anger & Dawirs,

271   1981), the zoeae I of the intertidal crab *Neohelice granulata* (Bas, Spivak & Anger, 2008), the

272   phyllosoma I of *Panulirus cygnus* (Liddy, Phillips & Maguire, 2003), and stage JIII of the

273   crayfish *Cherax quadricarinatus* (Stumpf et al., 2010).

274   The percent ratio of the PNR to the JI stage duration of individuals from the FC was 66%

275   in the warm season and 39% in the cold season. Therefore, JIs of *P. argus* would appear to be

276   more resistant to starvation during the warm season, consistent with the optimum temperature of

277   ~30°C for development of this stage (Lellis & Russell, 1990). This finding has implications for

278   *P. argus* in the context of climate change. For example, in the packhorse lobster *Sagmariasus*

279   *verreauxii*, a species that lives in waters ranging in temperature from 12 to 28 °C, the upper pejus

280   range of the puerulus is between 24 and 27° C, with a significant decrease in metabolic rates

281   between 27 and 30°C (Fitzgibbon et al. 2014b). To our knowledge, the thermal tolerance of the

282   puerulus or JIs of *P. argus* has not been determined, but Lellis & Russel (1990) found that, after

283   10 weeks in the laboratory, JIs of *P. argus* reared abt 33 °C had lower survival, smaller molting

284   increments, and lower feed conversion than JIs reared at 30°C. This finding would appear to

285   suggest that 33°C is beyond the upper pejus range of the early benthic juveniles of *P. argus*.

286   Evidence from several studies suggests that the species of marine ectotherms that would be

287   impacted the most with increased warming would be those from tropical areas because they are

288   already living close to their upper temperature limits (e.g. Silliman, 2003; Nguyen et al. 2011).

289   Although most species have some phenotypic plasticity for environmental tolerance (e.g. Kelly

290   et al., 2012), this issue also remains to be determined for *P. argus* postlarvae and small juveniles.

291   Despite the commercial importance of *P. argus*, the present study is the first to address

292   resistance to starvation in the early benthic juveniles of this species after they have undergone a

293   protracted lecithotrophic stage that may lead to their return to coastal waters from distances of up

294   to tens of kilometers offshore. Determination of the resistance to starvation of early juveniles of

295   this and other spiny lobsters could prove useful not only through its contribution to the

296   knowledge of biological aspects of life cycles, but also through provision of information that

297   could lead to the establishment of successful rearing protocols for aquaculture or to the

298   generation of predictive models for capture.

299

300   **ACKNOWLEDGEMENTS**

301   The authors wish to acknowledge the invaluable technical assistance provided by F.

302 Negrete-Soto and C. Barradas-Ortiz in the set-up of experiments and throughout the study. J.P.

303 Huchin-Mian, R. Martínez-Calderón, R. Candia-Zulbarán, R. Muñoz de Cote-Hernández, L. Cid-

304 González, and H. Canizales-Flores provided additional help. We also thank Ann Grant for her

305 assistance in reviewing the English.

306

307 **REFERENCES**

308 Abrunhosa FA, Kittaka J. 1997. Effect of starvation on the first larvae of *Homarus americanus*

309   (Decapoda, Nephropidae) and phyllosomas of *Jasus verreauxi* and *J. edwardsii* (Decapoda,

310   Palinuridae). *Bulletin of Marine Science* 61:73–80.

311 Aguilar W, Arceo-Briseño P, González-Cano J, Medina AI, Ríos-Lara V, Sosa-Cordero E,

312   Wakida-Kusonoki A. 2003. La pesquería de langosta en Yucatán y Quintana Roo, México.

313   *FAO Fisheries Report* 715:119–130.

314 Anger K. 1987. The D_0 threshold: a critical point in the larval development of decapod

315   crustaceans. *Journal of Experimental Marine Biology and Ecology* 108:15–30 DOI

316   10.1016/0022-0981(87)90128-6.

317 Anger K. 1995. Starvation resistance in larvae of a semiterrestrial crab, *Sesarma curaçaoense*

318   (Decapoda: Grapsidae). *Journal of Experimental Marine Biology and Ecology* 187:161–174

319   DOI 10.1016/0022-0981(94)00178-G.

320 Anger K. 2001. *The biology of decapod crustacean larvae*. Lisse: Balkema.

321 Anger K, Dawirs RR. 1981. Influence of starvation on the larval development of *Hyas araneus*

322   (Decapoda, Majidae). *Helgoländer Meeresuntersuchungen* 34:287–311 DOI

323   10.1007/BF02074124.

324   Anger K, Dawirs RR, Anger V, Goy JW, Costlow JD. 1981. Starvation resistance in first stage

325   zoeae of brachyuran crabs in relation to temperature. *Journal of Crustacean Biology* 1:518–

326   525 DOI 10.1163/1937240X81X00348.

327   Bas CC, Spivak E, Anger K. 2008. Variation in early developmental stages in two populations of

328   an intertidal crab, *Neohelice (Chasmagnathus) granulata*. *Helgoland Marine Research*

329   62:393–401 DOI 10.1007/s10152-008-0128-5.

330   Blaxter JHS, Hempel G. 1963. The influence of egg size on herring larvae (*Clupea harengus* L.).

331   *Journal du Conseil International pour l'Exploration de la Mer* 28:211–240 DOI

332   10.1093/icesjms/28.2.211.

333   Briones P, Lozano E, Cabrera MA, Arceo P. 1997. Biología y ecología de las langostas

334   (Crustacea: Decapoda: Palinuridae). In: Flores-Hernández D, Sánchez-Gil P, Seijo JC,

335   Arreguín-Sánchez F, eds. *Análisis y diagnóstico de los recursos pesqueros críticos del Golfo*

336   *de México*. Campeche: EPOMEX-Serie Científica 7, 81–99.

337   Briones-Fourzán P. 1994. Variability in postlarval recruitment of the spiny lobster *Panulirus*

338   *argus* (Latreille, 1804) to the Mexican Caribbean coast. *Crustaceana* 66:326–340 DOI

339   10.1163/156854094X00062.

340   Briones-Fourzán P, Lozano-Álvarez E. 2001. The importance of *Lobophora variegata*

341   (Phaeophyta: Dictyotales) as a habitat for small juveniles of *Panulirus argus* (Decapoda:

342   Palinuridae) in a tropical reef lagoon. *Bulletin of Marine Science* 68:207–219.

343   Briones-Fourzán P, Candela J, Lozano-Álvarez E. 2008. Postlarval settlement of the spiny

344   lobster *Panulirus argus* along the Caribbean coast of Mexico: patterns, influence of physical

345   factors, and possible sources of origin. *Limnology and Oceanography* 53:970–985 DOI

346   10.4319/lo.2008.53.3.0970.

347   Brito R, Díaz-Iglesias E, Rodríguez E, Conceiçao RM. 1991. Metabolismo energético de

348   postlarvas de langosta *Panulirus argus* sometidas a diferentes condiciones experimentales.

349   *Revista de Investigaciones Marinas* 12:312–322.

350   Butler MJ, Herrnkind WF. 2000. Puerulus and juvenile ecology. In: Phillips BF, Kittaka J, eds.

351   *Spiny lobsters: fisheries and culture, 2nd edn*. Oxford: Blackwell, 276–301.

352   Calado R, Pimentel T, Pochelon P, Olaguer-Feliú AO, Queiroga H. 2010. Effect of food

353   deprivation in late larval development and early benthic life of temperate marine coastal and

354   estuarine caridean shrimp. *Journal of Experimental Marine Biology and Ecology* 384:107–

355   112 DOI 10.1016/j.jembe.2010.01.003.

356   Calvo NS, Tropea C, Anger K, López-Greco LS. 2012. Starvation resistance in juvenile

357   freshwater crayfish. *Aquatic Biology* 16:287–297 DOI 10.3354/ab00451.

358   Cox SL, Jeffs A, Davies M. 2008. Developmental changes in the mouthparts of juvenile

359   Caribbean spiny lobster, *Panulirus argus*: Implications for aquaculture. *Aquaculture* 283:168–

360   174 DOI 10.1016/j.aquaculture.2008.07.019.

361   Díaz-Iglesias E, Robles-Murillo AK, Buesa RJ, Báez-Hidalgo M, López-Zenteno M. 2011.

362   Bioenergetics of red spiny lobster *Panulirus interruptus* (Randall, 1840) juveniles fed with

363   mollusc. *Aquaculture* 318:207–218 DOI 10.1016/j.aquaculture.2011.05.008.

364   Ehrhardt N, Puga R, Butler M. 2011. Implications of the ecosystem approach to fisheries

365   management in large ecosystems: the Caribbean spiny lobster, *Panulirus argus*, fisheries as a

366   case. In: Fanning L, Mahon R. McConney P, eds. *Towards marine ecosystem-based*

367   *management in the wider Caribbean*. Amsterdam: Amsterdam University Press, 157–175.

368   Figueiredo J, Penha-Lopes G. Narciso L, Lin J. 2008. Effect of starvation during late megalopa

369   stage of *Mithraculus forceps* (Brachyura: Majidae) on larval duration, synchronism of

370   metamorphosis, survival to juvenile, and newly metamorphosed juvenile size. *Aquaculture*

371   274:175–180 DOI 10.1016/j.aquaculture.2007.10.052.

372 Fitzgibbon QP, Jeffs AG, Battaglene SC. 2014a. The Achilles heel for spiny lobsters: the

373   energetics of the non-feeding post-larval stage. *Fish and Fisheries* 15:312–326 DOI

374   10.1111/faf.12018.

375 Fitzgibbon QP, Ruff N, Tracey SR, Battaglene SC. 2014b. Thermal tolerance of the nektonic

376   puerulus stage of spiny lobsters and implications of ocean warming. *Marine Ecology Progress*

377   *Series* 515:173–186 DOI 10.3354/meps10979.

378 Gebauer P, Pashke K, Anger K. 2010. Seasonal variation in the nutritional vulnerability of first-

379   stage larval porcelain crab, *Petrolisthes laevigatus* (Anomura: Porcellanidae) in southern

380   Chile. *Journal of Experimental Marine Biology and Ecology* 386:103–112 DOI

381   10.1016/j.jembe.2010.02.016.

382 Glencross B, Smith M, Curnow J, Smith D, Williams K. 2001. The dietary protein and lipid

383   requirements of post-puerulus western rock lobster, *Panulirus cygnus*. *Aquaculture* 199:119–

384   129 DOI 10.1016/S0044-8486(01)00520-8.

385 Goldstein JS, Matsuda H, Takenouchi T, Butler MJ. 2008. The complete development of larval

386   Caribbean spiny lobster *Panulirus argus* (Latreille, 1804) in culture. *Journal of Crustacean*

387   *Biology* 28:306–327 DOI 10.1651/0278-0372(2008)028[0306:TCDOLC]2.0.CO;2.

388 Guerao G, Simeó CG, Anger K, Urzúa Á, Rotllant G. 2012. Nutritional vulnerability of early

389   zoea larvae of the crab *Maja brachydactyla* (Brachyura, Majidae). *Aquatic Biology* 16:253–

390   264 DOI 10.3354/ab00457.

391 Gutiérrez-Carbonell D, Simonín-Díaz J, Briones-Fourzán P. 1992. A simple collector for

392   postlarvae of the spiny lobster *Panulirus argus*. *Proceedings of the Gulf and Caribbean*

393   *Fisheries Institute* 41:516–527.

394 Gutow L, Leidenberger S, Boos K, Franke HD. 2007. Differential life history responses of two

395   *Idotea* species (Crustacea: Isopoda) to food limitation. Marine Ecology Progress Series

396   344:159–172 DOI 10.3354/meps06894.

397 Harms J. 1992. Larval development and delayed metamorphosis in the hermit crab *Clibanarius*

398   *erythropus* (Latreille) (Crustacea, Diogenidae). *Journal of Experimental Marine Biology and*

399   *Ecology* 156:151–160 DOI 10.1016/0022-0981(92)90242-3.

400 Harms J, Moal J, Le Coz JR, Daniel JY, Samain JF. 1990. Nucleotide composition and energy

401   charge in growing and starving zoea I of *Carcinus maenas* (Decapoda: Portunidae).

402   *Comparative Biochemistry and Physiology B* 96:405–414 DOI 10.1016/0305-

403   0491(90)90396-B.

404 Holthuis LB. 1991. *Marine lobsters of the world*. FAO Species Catalogue 13. Rome: FAO.

405 Jeffs AG, Willmott ME, Wells RMG. 1999. The use of energy stores in the puerulus of the spiny

406   lobster *Jasus edwardsii* across the continental shelf of New Zealand. *Comparative*

407   *Biochemistry and Physiology A* 123:351–357 DOI 10.1016/S1095-6433(99)00073-2.

408 Jeffs AG, Chiswell SM, Booth JD. 2001. Distribution and condition of pueruli of the spiny

409   lobster *Jasus edwardsii* offshore from north-east New Zealand. *Marine and Freshwater*

410   *Research* 52:1211–1216 DOI 10.1071/MF01182.

411 Kelly MW, Sanford E, Grosberg RK (2012) Limited potential for adaptation to climate change in

412   a broadly distributed marine crustacean. *Proceedings of the Royal Society B* 279:349−356

413   DOI 10.1098/rspb.2011.0542.

414 Kittaka J. 1994. Culture of phyllosomas of spiny lobster and its application to studies of larval
415   recruitment and aquaculture. *Crustaceana* 66:258–270 DOI 10.1163/156854094X00026.

416 Lellis WA, Russell JA. 1990. Effect of temperature on survival, growth and feed intake of
417   postlarval spiny lobsters, *Panulirus argus*. *Aquaculture* 90:1–9 DOI 10.1016/0044-
418   8486(90)90277-T.

419 Lemmens JWTJ. 1994. Biochemical evidence for absence of feeding in pueruli larvae of the
420   western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae). *Marine Biology* 118:383–391
421   DOI 10.1007/BF00350295.

422 Lewis JB, Moore HB, Babis W. 1952. The post-larval stages of the spiny lobster *Panulirus*
423   *argus*. *Bulletin of Marine Science of the Gulf and Caribbean* 2:324–337.

424 Liddy GC, Phillips BF, Maguire GB. 2003. Survival and growth of instar 1 phyllosoma of the
425   western rock lobster, *Panulirus cygnus*, starved before or after periods of feeding.
426   *Aquaculture International* 11:53–67 DOI 10.1023/A:1024100110378.

427 Limbourn AJ, Babcock RC, Johnston DJ, Nichols PD, Knott B. 2008. Post-settlement energy
428   reserves in *Panulirus cygnus*: experimental effects of starvation on survival and nutritional
429   condition. *Marine Biology* 153:445–456 DOI 10.1007/s00227-007-0825-7.

430 Lozano-Álvarez E. 1996. Ongrowing of juvenile spiny lobsters, *Panulirus argus* (Latreille,
431   1804) (Decapoda, Palinuridae), in portable sea enclosures. *Crustaceana* 69:958–973 DOI
432   10.1163/156854096X00402.

433 McWilliam PS, Phillips BF. 1997. Metamorphosis of the final phyllosoma and secondary
434   lecithotrophy in the puerulus of *Panulirus cygnus* George: a review. *Marine Freshwater*
435   *Research* 48:783–790 DOI 10.1071/MF97159.

436 Mikami S, Greenwood JG, Gillespie NC. 1995. The effect of starvation and feeding regimes on

437   survival, intermoult period and growth of cultured *Panulirus japonicus* and *Thenus* sp.

438   phyllosomas (Decapoda, Palinuridae and Scyllaridae). *Crustaceana* 68:160–169 DOI

439   10.1163/156854095X01312.

440 Nguyen KDT, Morley SA, Lai CH, Clark MS, Tan KS, Bates AE, Peck LS (2011) Upper

441   temperature limits of tropical marine ectotherms: global warming implications. *PLoS ONE*

442   6:e29340 DOI 10.1371/journal.pone.0029340

443 Pantaleão JAF, Barros-Alves SP, Tropea C, Alves DFR, Negreiros-Fransozo ML, López-Greco

444   LS. 2015. Nutritional vulnerability in early stages of the freshwater ornamental "red cherry

445   shrimp" *Neocaridina davidi* (Bouvier, 1904) (Caridea: Atyidae). *Journal of Crustacean*

446   *Biology* 35:676–681 DOI 10.1163/1937240X-00002357.

447 Paschke K, Gebauer P, Buschholz F, Anger K. 2004. Seasonal variation in starvation resistance

448   of early larval North Sea shrimp *Crangon crangon* (Decapoda: Crangonidae). *Marine*

449   *Ecology Progress Series* 279:183–191 DOI 10.3354/meps279183.

450 Perera E, Díaz-Iglesias E, Fraga I, Carrillo O, Galich GS. 2007. Effect of body weight,

451   temperature and feeding on the metabolic rate in the spiny lobster *Panulirus argus* (Latreille,

452   1804). *Aquaculture* 265:261–270 DOI 10.1016/j.aquaculture.2006.11.012.

453 Phillips BF, Cruz R, Caputi N, Brown RS. 2000. Predicting the catch of spiny lobster fisheries.

454   In: Phillips BF, Kittaka J, eds. *Spiny lobsters: fisheries and culture, 2nd edn*. Oxford:

455   Blackwell, 357–375.

456 Phillips BF, Melville-Smith R, Kay MC, Vega-Velázquez A. 2013. *Panulirus* species. In:

457   Phillips BF, ed. *Lobsters: biology, management, aquaculture, and fisheries, 2nd edn*. Oxford:

458   Wiley-Blackwell, 289–325.

459   Phillips BF, Booth JD, Cobb JS, Jeffs AG, McWilliam P. 2006. Larval and postlarval ecology.

460   In: Phillips BF, ed. *Lobsters: biology, management, aquaculture, and fisheries*. Oxford:

461   Blackwell, 231–262.

462   Smith DM, Williams KC, Irvin SJ. 2005. Response of the tropical spiny lobster *Panulirus*

463   *ornatus* to protein content of pelleted feed and to a diet of mussel flesh. *Aquaculture Nutrition*

464   11:209–217 DOI 10.1111/j.1365-2095.2005.00344.x.

465   Stillman JH. 2003. Acclimation capacity underlies susceptibility to climate change. *Science*

466   301:65 DOI: 10.1126/science.1083073.

467   Stumpf L, Calvo NS, Pietrokovsky S, López-Greco LS. 2010. Nutritional vulnerability and

468   compensatory growth in early juveniles of the "red claw" crayfish *Cherax quadricarinatus*.

469   *Aquaculture* 304:34–41 DOI 10.1016/j.aquaculture.2010.03.011.

470   Sulkin SD. 1978. Nutritional requirements during larval development of the portunid crab,

471   *Callinectes sapidus* Rathbun. *Journal of Experimental Marine Biology and Ecology* 34:29–41

472   DOI 10.1016/0022-0981(78)90055-2.

473   Ventura T, Fitzgibbon QP, Battaglene SC, Elizur A. 2015. Redefining metamorphosis in spiny

474   lobsters: molecular analysis of the phyllosoma to puerulus transition in *Sagmariasus*

475   *verreauxi*. *Scientific Reports* 5:13537 DOI 10.1038/srep13537.

476   Weiss HM, Lozano-Álvarez E, Briones-Fourzán P. 2008. Circadian shelter occupancy patterns

477   and predator-prey interactions of juvenile Caribbean spiny lobsters in a reef lagoon. *Marine*

478   *Biology* 153:953–963 DOI 10.1007/s00227-007-0867-x.

479   Witham R. 1973. Preliminary thermal studies on young *Panulirus argus*. *Florida Scientist* 36:

480   154–158.

481

Figure 1

Experimental Design of PNR and PRS.

Design of point-of-no-return (PNR; initial starvation, followed by feeding) and point-of-reserve-saturation (PRS; initial feeding, followed by starvation) experiments. FC: continuously fed control; SC: continuously starved control; S3–S18: no. of days of starvation, followed by continuous feeding; F3–F18: no. of days of feeding, followed by continuous starvation. Initial number of *Panulirus argus* pueruli per treatment, FC and SC groups, *n* = 20.
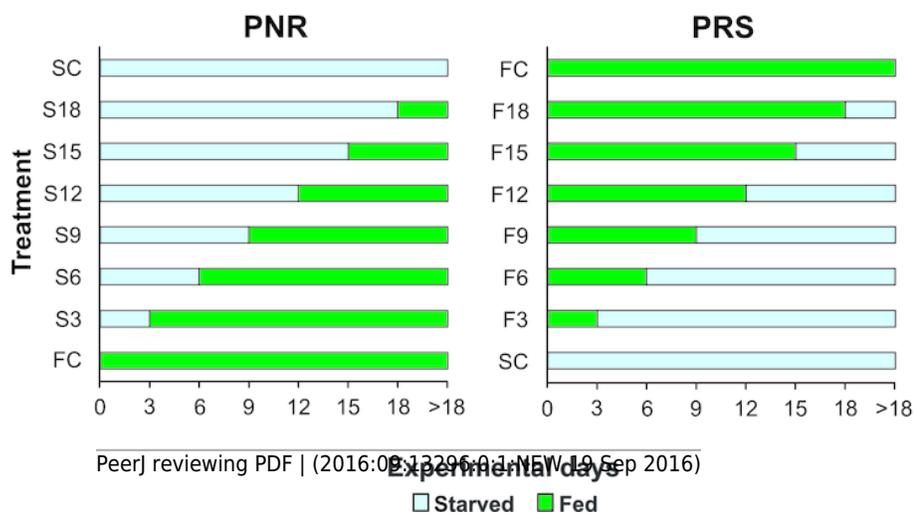
Figure 2

Intermolt period in PNR experiments.

Intermolt period (days) between JI and JII stages of *Panulirus argus* subjected to different periods of starvation followed by continuous feeding in experiments to determine the point-of-no-return. Red columns, warm season, June-October 2012; blue columns, cold season, January-April 2013. Results from treatments with two survivors or fewer were omitted. Error bars, 95% CI.
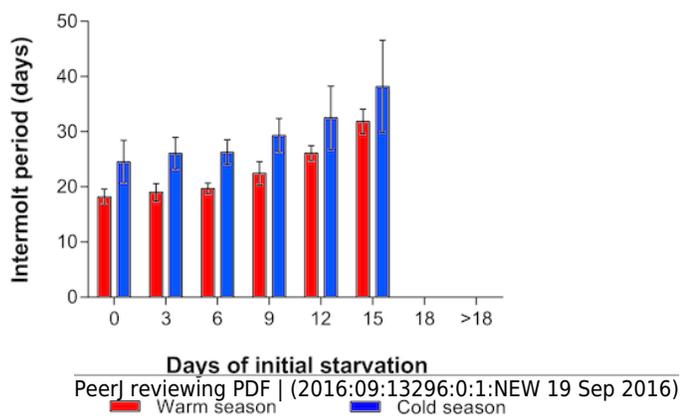
Figure 3

Sigmoidal dose-response curves - PNR experiments.

Sigmoidal dose-response curves fitted to the mortality (%) of JIs of *Panulirus argus* subjected to a period of starvation before being fed. Triangles, warm season, June-October 2012; circles, cold season, January-April 2013. PNR_{50} values: 12.1 ± 1.2 d in the warm season and 9.5 ± 2.1 d in the cold season.
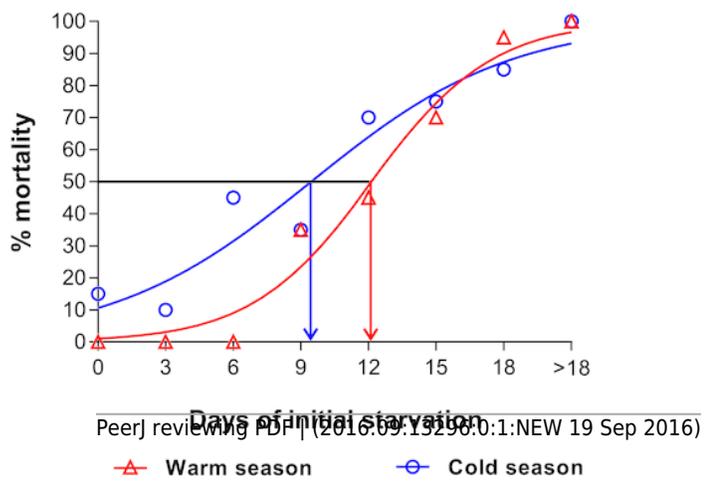
Figure 4

Intermolt period in PRS experiments.
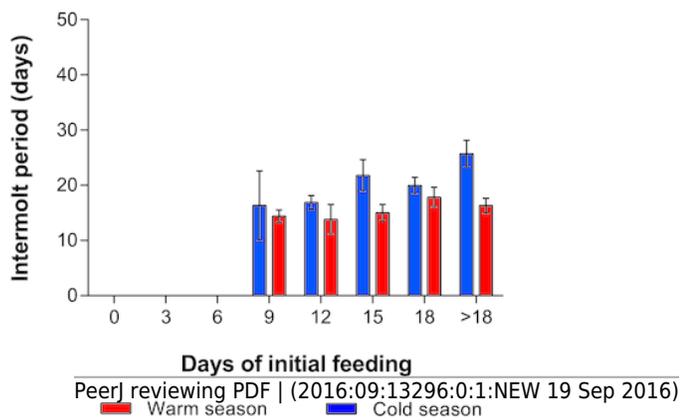
Figure 5

Sigmoidal dose-response curves - PRS experiments.

Sigmoidal dose-response curves fitted to the mortality (%) of JIs of *Panulirus argus* allowed periods of feeding before being starved. Circles, cold season, January-April 2014; triangles, warm season, July-November 2014. PRS_{50} values: 13.1 ± 0.7 d in the cold season and 12.1 ± 1.1 d in the warm season.