Vol. 33, Nº 2, julio-diciembre, 2023

ISSN L 2710 - 7647

https://revistas.up.ac.pa/index.php/scientia/

pp. 31-62

EFECTO DE ETIQUETAS PIT EN LA CONDICIÓN DE *Cardisoma crassum* (BRACHYURA: GECARCINIDAE) EN CAUTIVERIO, DE HICACO, VERAGUAS, PANAMÁ

PIT TAG EFFECT ON CAPTIVE *Cardisoma crassum* (BRACHYURA: GECARCINIDAE) CONDITION, FROM HICACO, VERAGUAS, PANAMA

Roberto Carlos Lombardo González

Universidad de Panamá, Centro de Capacitación, Investigación y Monitoreo de la Biodiversidad,

Centro Regional Universitario de Veraguas. Panamá.

https://orcid.org/0000-0002-0279-8621

roberto.lombardo@up.ac.pa

DOI https://doi.org/10.48204/j.scientia.v33n2.a4057

Recepción	**Aprobación**
17/02/2023	31/05//2023

Resumen

La trazabilidad en acuicultura y estudios de campo requiere identificación confiable de los individuos. Las etiquetas internas pueden ayudar a mantener registros de identidad y evitar la pérdida típica de etiquetas externas debido a la muda en los crustáceos. Aquí presentamos el primer intento de implantar etiquetas de transpondedor integrado pasivo (PIT) en el cangrejo azul (*Cardisoma crassum*), en cautiverio, para evaluar su efecto en el estado de salud y su influencia en el peso y la mortalidad. Adicionalmente, la temperatura durante el experimento en cautiverio fue registrada para evaluar su efecto en la condición de los cangrejos. Las etiquetas PIT se implantaron por inyección en la base del cuarto pereiópodo. El cangrejo marcado más pequeño fue de 55,26 mm de ancho de cefalotórax; todos los cangrejos retuvieron los implantes hasta el término. La supervivencia fue del 77,8 % y no hubo diferencias en el peso y en el factor de condición como resultado de la implantación de la etiqueta. El deterioro de la condición del cangrejo se correlacionó con el estrés térmico. Un mayor desarrollo de la acuicultura debe controlar el estrés térmico en cautiverio para reducir la mortalidad. Las etiquetas PIT sirvieron como un medio confiable para la identificación individual de cangrejos azules.

Palabras clave: Cangrejo azul, cefalotórax, estrés por calor, sobrevivencia, peso

Abstract

Traceability in aquaculture and field studies requires reliable individual identification. Internal tags can aid identity record keeping and prevent typical external tag loss due to molting in Crustaceans. Here we present the first attempt at implanting passive integrated transponder (PIT) tags in the blue crab (*Cardisoma crassum*), in captivity, to evaluate their effect on general condition, weight, and mortality. Additionally, temperature records were logged during the experiment to evaluate its effect on crab condition. PIT tags were implanted by injection through the base of the fourth pereiopod. All crabs retained tags to term, and the smallest surviving tagged crab was 55.26 mm in cephalothorax width. Survival was 77.8%, and there was no difference in weight or condition factor as a result of tag implantation. Deteriorating crab condition was correlated with heat stress. Further aquaculture development must control heat stress in captivity to reduce mortality. PIT tags served as a reliable means for individual blue crab identification.

Keywords: Blue crab, cephalothorax, heat stress, survival, weight

Introduction

Accurate individual identification is key for aquaculture research and production farms (Foote et al., 2018; Fuller and McEntire, 2013; Rasal et al., 2021). Several techniques have been used to tag individual animals for the purpose of studying their movement, habitat use, behavior, physiology and survival (Haddaway et al., 2011; Lauzon-Guay and Scheibling, 2008; Lombardo and Rojas, 2022).

In crustaceans, several external and internal tagging methods have been developed for the accurate identification of individuals (Haddaway et al., 2011; Sato et al., 2020). Such methods have been applied to study behavior (Drew et al., 2012; Rondeau and Sainte-Marie, 2016), population density (Skurdal et al., 1992), migration (Smith et al., 2001), site fidelity (Forsee and Albrecht, 2012; Goshima et al., 1978; Moraes-Costa and Schwamborn, 2018) and growth (Sato et al., 2013, 2020; Ulmestrand and Eggert, 2001).

External tagging in crustaceans has limitations due to periodic moulting (Mclay, 2015), where individuals discard the exoskeleton, which may result in loss of external tags (Haddaway et al., 2011; Sato et al., 2020). In contrast, internal tagging methods show high retention rates with minimal drawbacks (Sato et al., 2013), and do not show detrimental effects in the individual (Foote et al., 2018; Fuller and McEntire, 2013; Haddaway et al., 2011; Moraes-Costa and Schwamborn, 2018). Furthermore, internal tagging methods in crustaceans include microwire tags, (Sharp et al., 2000), visible elastomer implants and alphanumeric internal tags (Dinh et al., 2012; Haddaway et al., 2011), as well as passive integrated transponder (PIT) tags (Drew et al., 2012; Forsee and Albrecht, 2012; Haddaway et al., 2011; Meynecke et al., 2015; Moraes-Costa and Schwamborn, 2018; Sato et al., 2013).

PIT tags consist of an electronic microchip encased in a biocompatible material, (e.g., glass or surgical grade plastic). Transponders can be programmed with an infinite number of unique codes, do not require battery, and thus have unlimited life expectancy (Drew et al., 2012). Through a scanner, the distinctive code embedded in PIT tags can be read when energized by an electromagnetic field. This allows detecting and identifying animals carrying tags, without destructive sampling (Sato et al., 2013). Tags are placed internally via injection, therefore the risk of tag loss at moulting is significantly reduced compared with external tags (Moraes-Costa and Schwamborn, 2018).

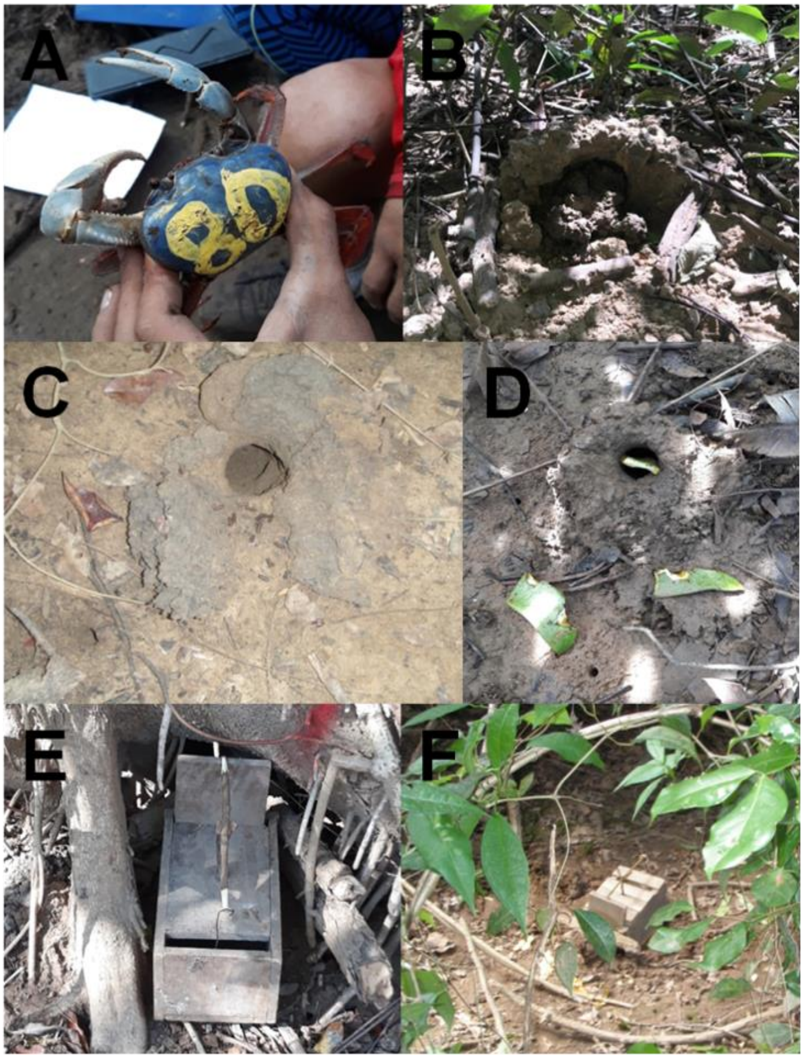
The Blue Crab *Cardisoma crassum*, Smith 1870 is an important fishery resource and common resident of mangrove ecosystems in the Panamanian Pacific (Lombardo and Rojas, 2022; Vega et al., 2018). To date, aquaculture of *C. crassum* remains undeveloped in the country and there are only two published studies on *C. crassum* from Panama. Vega et al., (2018) described the blue crab artisanal fishery, while Lombardo and Rojas (2022) provided the first report of burrow fidelity in *C. crassum*, from the northeastern side of the Montijo Gulf. In the latter, burrow fidelity was evaluated with a mark-recapture method using an external mark of enamel paint

(Fig. 1A). This allowed tracking of individuals for a limited time period that ended with the onset of moulting and/or when individuals sealed themselves inside burrows (Fig. 1B) by placing a mud plug at the burrow entrance (Lombardo and Rojas, 2022). The above scenario implies that studies requiring extended follow-up of individuals in captivity or in the wild might be limited because the identity of post-moult and reemerging blue crabs (externally marked) is uncertain due to mark loss or burrow ownership changes prior to burrow plugging (Lombardo and Rojas, 2022). In this context, internally implanted PIT tags would allow accurate individual identity tracking over long time periods without the risk associated with external tag loss.

In Panama, the fishery of blue crab is not regulated (A. Vega, per. commun., 2020). This generates urgency for research where the identity and fate of individuals is tracked in studies spanning multiple seasons. This can be achieved by PIT tagging, to shed light over unresolved behavioral ecology and life history traits of the blue crab, including resource management and aquaculture development. For example, reliable identity tracking is necessary in advanced breeding programs under targeted mating based on pedigree and/or other traits such as disease loading (Foote et al., 2018; Rasal et al., 2021). Also, follow-up of individual changes in behavior across seasons might be useful for resource management because changes in behavior can affect the vulnerability of a species to overharvesting (Moraes-Costa and Schwamborn, 2018; Schwamborn and Moraes-Costa, 2021); thus, accuracy in tracking individual identity is of paramount importance.

In light of the above, and considering PIT tagging has not been attempted in this species, our main objective was to test tag retention and evaluate its effect on *Cardisoma crassum* overall condition in order to determine its feasibility for use in future research to learn more about this valuable fishery resource.

Figure 1.
Cardisoma crassum external marking method and sampling criteria. A. Enamel paint over cephalothorax for individual identification. B. Burrow mud plug at entrance. C, D. Feces pellets outside burrow entrance and Avicennia germinans fruit remains as signs of crab activity. E, F. Wooden artisanal traps for sampling.



Methods

Sampling protocol

The experiment, conducted in captivity, employed crabs sampled from Hicaco (7.64944°N -81.19967°E), in Soná, Veraguas, from February to March 2022. To acquire the sample, burrows in the field were selected based on visual confirmation of occupation and recent activity signs at the entrance. Burrow occupation signs include freshly excavated mud outside the burrow, fresh feces pellets and/or fresh plant remains on entrance (Fig. 1C, D). When a burrow met these criteria, a wooden trap was set (Fig. 1E, F) to capture crabs. Once trapped, the sex of crabs was determined by the shape of the abdomen (Fischer et al., 1995), and if females were carrying eggs, they were excluded from the sample and released. This precautionary measure was taken to avoid potential confounding effects arising from factors such as mortality risk and significant weight fluctuations resulting from dislodging of the egg mass. Crabs missing no more than two pereiopods, chelae or a combination of both were deemed acceptable. Immobilized individuals were placed in lidded containers with water saturated sponges for transportation to the holding facility in Santiago Veraguas (Fig. 2A), no longer than 48 hours after capture.

Experimental settings in captivity

On arrival to the holding facility, crabs with evident signs of lethargy were excluded from the experiment. Crabs were held by the sides of the carapace (Fig. 3) and their sex and handedness (side of major chela) were verified, while biometric variables such as, cephalothorax width (CW), cephalothorax length (CL), chela height (QH) and chela length (QL) were measured with a digital vernier caliper (0.1 mm). As crabs exhibit a highly agitated response to handling, individuals were first marked by applying quick-drying white enamel to their cephalothorax. Subsequently, the crabs were weighed by placing them inside a plastic container on a digital scale, accurate to 0.01 grams. Once the measurement was recorded, the crabs were promptly released into their designated holding tank without any further physical contact.

Crabs were sorted and placed inside 23 L capacity lidded tanks, with 100 ml of brackish water and provided with clay pots as shelter. Tanks were raised above ground in wooden pallets (1 m x 1.20 m x 0.16 m) under the shade (Fig. 2A, B), where external, as well as temperature inside holding tanks was recorded (February–April) with data loggers (AZ 88163). The diet consisted of yuca (*Manihot esculenta*), maize (*Zea mays*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), mango (*Mangifera indica*) and coconut (*Cocos nucifera*). Holding tanks were cleaned and food items offered *ad libitum* at 48-hour intervals and regular photoperiod.

Figure 2.
Experimental settings for PIT tag effect assessment in Cardisoma crassum. A. Crab holding tanks (23 L), equipped with weighted lids for secure closure. Ventilation was allowed by placing a 4 cm wooden spacer under the lid. B. Internal holding conditions. Crabs were provided with clay pots for shelter; food items were placed in front of individuals.

Tag implantation

After an acclimation period of three days, the condition of crabs was evaluated using Mayze et al., (2014) liveliness index followed by tag implantation, where in the “very slow" state, the crabs are nearing death, characterized by legs that offer no resistance to external force and non-responsive pincers. The response to touch of the eye stalks is negligible, and there may be signs such as drooping mouth parts and foaming from the mouth. In the "slow" state, the subject displays slow movement of its legs with slight resistance to force, while the chelae exhibit a slow response; the eye stalk response is also diminished. Conversely, in the "lively" state, the legs of crabs exhibit quick movement with resistance to force, and the pincers are strong and active; eye stalks show a fast response to the touch. Finally, in the "very lively" state, the subject's legs demonstrate strength, with precise and forceful digging of the tips. The chelae are notably active and aggressive, while the eye stalk response is rapid; crabs might lunge at objects in a threat display. Prior to tag injection, crabs were submerged in an ice water bath (16–18 C°) until sedated (90–120 seconds) to reduce stress and facilitate the procedure. PIT tags type FDX-B (Biomark Mini HPT8, dimensions: 8 mm x 1.4 mm) were implanted into the base of the fourth pereiopod of crabs (Moraes-Costa and Schwamborn, 2018) by means of injection (Biomark MK165 Implanter syringe) with a 16-gauge needle (Fig. 3A, B). The injection was located in either side (right or left) of the crab avoiding the site of missing pereiopods or budding scars. The 15-digit ID number of tags was detected at close range with a portable pet microchip scanner (Smoostart 134.2 kHz) (Fig. 3C, D).

Once implanted the ID number of each individual was confirmed with the scanner and then returned to their respective holding tanks. Survival, tag retention and liveliness (Mayze et al., 2014) were evaluated at 48-hour intervals during 33 days by ocular inspection, scanner readings and display of normal signs of activity, such as startle reflex, rising chelae, aggressive lunging of the chelae and body, and overall escape response to handling.

Data analysis

The sex ratio of individuals in the sample was evaluated with the Chi-square goodness of fit test. Biometric data was sorted and tabulated for standard descriptive purposes. Length and weight differences between sexes were assessed with the Student t-test. The relationship between biometric variable predictors CL, QH, QL, TW and CW (response) was analyzed with regression (least squares). The objective of the analysis was to construct a model that could identify the most suitable predictor(s) for CW (Dixon and Massey, 1957). This approach was adopted due to the significance of CW as a proxy measure for assessing most crab fisheries.

The model was fitted by stepwise process ($\alpha = 0.15$), according to the P-value of terms and predictor significance diagnostic by Pareto standardized effects plot. Weight was used as a proxy for general crab condition and indicator of tag effect. The weight (g) of crabs was measured at four stages within the 33-day experimental period and later compared with one-way ANOVA. Crabs were weighted in the third day after the acclimation period, and then after PIT tag implantation at 17, 26 and 31 days.

Figure 3.

Tag implantation procedure in Cardisoma crassum. A. Biomark Mini HPT8 PIT tag measuring 8 mm x 1.4 mm. B. Tag detail with antenna copper wire coil and casing. C. Tag implantation with applicator syringe and 16-gauge needle to the base of the fourth pereiopod. D. Close range scanner for PIT tag detection. E. Scanner display screen with 15-digit ID number.

Individuals were monitored every 48 hours and the survival-mortality proportion recorded. To compare these proportions, a hypothesis test was run using the difference in said proportions along a 95% confidence interval (C.I.) and corresponding P-value. The consistency of the observed mortality proportion is of importance to determine if it is acceptable; at least not to exceed the upper bound of a 95% confidence interval. Such interval including the observed population proportion was calculated and its upper bound used as cut-off value for the hypothesis Z-test. This particular test sought to determine if the observed proportion would statistically exceed the hypothesized proportion, thus providing insight in the expected mortality as a result of PIT tagging. The effect of temperature over the proportions of crabs in each condition category of the lively index was explored with the Pearson correlation.

The relationship between cephalothorax width and weight (CW-TW) was estimated using the equation for the potential function (Table 1). Since the CW-TW is not linear, and weight variability increases as individual length increase, a log-10 transformation was used (Froese, 2006; Ogle, 2016; Ogle et al., 2021). The slopes were tested for their inclusion in the isometric (b = 3) or allometric growth range (negative allometric; b < 3 or positive allometric; b > 3) by computing a t-test and P-value (Dixon and Massey, 1957; Froese, 2006) with the equation:

$$t_s = \frac{(3 - b)}{S_b}$$

where the number three represents the cut-off value for when the null hypothesis is true, “b” is the observed slope value and S_b is its estimated standard error (from regression analysis). Furthermore, after data transformation, slopes and intercepts of cephalothorax width (CW) and weight (TW) were used to calculate three condition indexes, before and after tag implant. Fulton’s (*k*) condition index (Fulton, 1902), Ricker’s modified condition index (Ricker, 1975) and LeCren’s (k_n) relative condition index (Huxley, 1950; Le Cren, 1951), were used to infer the general status and effect of PIT tag implantation in our sample (Table 1). The resulting values before and after the implantation of PIT tags were compared, for each index, with the two-sample Student t-test.

It is unknown if confinement or sex-related differences may have an effect on crab stress levels that might produce limb loss. To explore whether these factors and limb loss had any connection, the proportion of complete individuals and those with missing limbs were counted; the Chi-square test was used to determine if there was significant difference between proportions. The weight gain of individuals was compared (Student t-test) to explore potential difference due to sex or limb loss. Data was organized using a spreadsheet in MS Excel and processed with Minitab19 statistical software.

Table 1.

Formulae for Cardisoma crassum cephalothorax width-weight relationship (CW-TW) and condition index (k). Letters "a" and "b" are the exponential forms of the intercept and slope, from the logarithmic length-weight equation (Le Cren, 1951), TW is the total weight and CW, the cephalothorax width.

Index	**Equation**		**Regression parameters**			
			Before PIT tag implant		**After PIT tag implant**	
Potential function	$\mathrm{TW} = \mathrm{a} \cdot \mathrm{CW}^{\mathrm{b}}$		**(*b*)**	**(*a*)**	**(*b*)**	**(*a*)**
Fulton's (*k*)	$\mathrm{k} = 1000 * (\mathrm{TW}/\mathrm{CW}^{3})$	Pooled	2.782	0.001164	2.778	0.001195
Ricker's	$\mathrm{k} = 1000 * (\mathrm{TW}/\mathrm{CW}^{\mathrm{b}})$	Male	2.761	0.001278	2.754	0.001327
LeCren's	$\mathrm{K_n} = \mathrm{TW}/(\mathrm{a} * \mathrm{CW}^{\mathrm{b}})$	Female	2.845	0.000894	2.867	0.000825

Results

Biometry

A total of 36 blue crabs were captured from February to March 2022 of which, 25 were males and 11 females (no gravid females found) with a statistically uneven sex ratio of 2.3:1 (Chi-square goodness of fit test, $\chi^2 = 5.44$, $P = 0.02$). Pooled mean CW was 67.63 ± 7.78 mm (54.56–83.42 mm, N = 36), while the average TW was 148.92 ± 49.51 g (76.41–284.79 g, N = 36). Male CW averaged 69.16 ± 8.11 mm (55.26–83.42 mm, N = 25) and 64.15 ± 5.91 mm for females (54.56–76.09 mm, N = 11) (Table 2). When comparing biometric variables between sexes, males were larger and heavier than females (CW, t = 2.08, d.f. = 26, P = 0.05; QL, t = 3.44, d.f. = 32, P = 0.002; QH, t = 2.27, d.f. = 26, P = 0.03; TW, t = 2.24, d.f. = 29, P = 0.03), except for CL where the means were not significantly different (Student t-test, t = 1.49, d.f. = 25, P = 0.15).

The relationship between the biometric variable predictors (CL, QH, QL and TW) and the response (CW) was confirmed in the regression model ($r^2 = 0.9716$, $F_{(2,33)} = 564.52$, $P < 0.001$). Model fitting by stepwise selection indicated that the best predictors for crab CW in our sample were CL ($r^2 = 0.9632$, $F_{(1,34)} = 352.28$, $P < 0.001$), TW ($r^2 = 0.9341$, $F_{(1,34)} = 482.19$, $P < 0.001$), and QL ($r^2 = 0.7743$, $F_{(1,33)} = 113.18$, $P = 0.004$), while QH did not contribute significantly to the fit ($r^2 = 0.4942$; Fig.4).

Figure 4.

Relationship between biometric variables in Cardisoma crassum from Hicaco, Soná, Veraguas, Panama Pacific. Cephalothorax width (CW), cephalothorax length (CL), chela height (QH), chela length (QL) and total weight (TW). Weight is reported in grams, while the rest of variables are in millimeters.

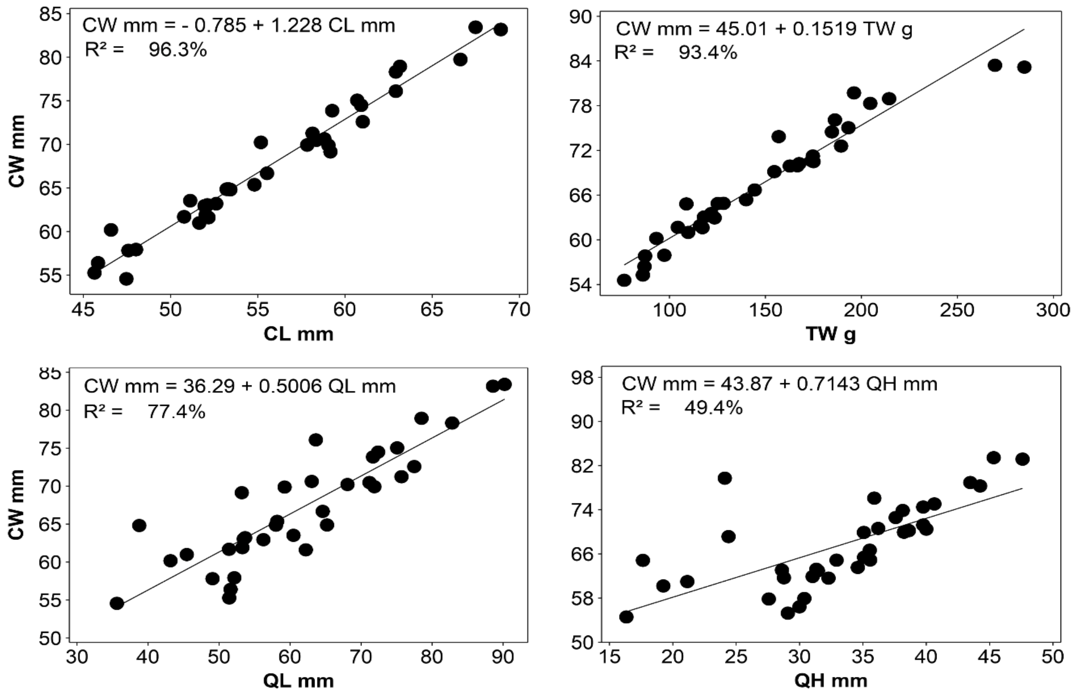


Table 2.

Cardisoma crassum descriptive statistics from Hicaco, Veraguas, Panama. Cephalothorax width (CW), cephalothorax length (CL), chela height (QH), chela length (QL), total weight (TW), and Standard deviation (SD); all in millimeters (mm)

Variable	**Mean ± SD**		**Minimum- Maximum**	
	♂	♀	♂	♀
CW	69.16 ± 8.11	64.15 ± 5.91	55.26-83.42	54.56-76.09
CL	56.63 ± 6.61	53.67 ± 4.89	45.63-68.93	47.46-62.91
QL	65.36 ± 13.74	53.36 ± 7.13	38.77-90.21	35.61-63.63
QH	34.88 ± 7.96	29.59 ± 5.63	17.63-47.6	16.33-35.9
TW	158.8 ± 52.9	126.47 ± 32.54	86.2-284.8	76.41-186.17

The cephalothorax width-weight relationship was significant for the pooled data, while in general, blue crabs showed allometric growth provided the slope was not significantly different from b = 3 (Student t-test, t = -2.13, df = 34, P = 0.040). However, when the slopes for each sex were tested separately, P-values larger than $\alpha = 0.05$ were obtained (♂ P = 0.087, ♀ P = 0.404), indicating isometric growth (Table 3).

Table 3.

Relationship between cephalothorax width and weight (CW-WR), and allometry test on Cardisoma crassum, from Hicaco, Veraguas, Panama. Cephalothorax width (CW) in millimeters, total weight (TW) in grams, intercept (a), slope (b) from equation.

CW-WR	Linear regression equation	r^2	t-test allometry (H_o: b = 3)	$TW = a \cdot L^b$
Pooled	log(TW) = -2.934 + 2.782·log(CW)	0.96, $P < 0.001$	t = -2.13, df = 34, P = 0.040	$0.00116 \cdot L^{2.782}$
♂	log(TW) = -2.893 + 2.761·log(CW)	0.95, $P < 0.001$	t = -1.79, df = 23, P = 0.087	$0.00128 \cdot L^{2.761}$
♀	log(TW) = -3.048 + 2.845·log(CW)	0.97, $P < 0.001$	t = -0.87, df = 9, P = 0.404	$0.00089 \cdot L^{2.845}$

The 40.63% of crabs in the sample had missing appendages (pereiopods or chela) from the field, where 31.25% of males and 9.38% of females were missing appendages. There was no association between crab sex and the proportion of complete to incomplete individuals (Chi-square test for independence, $\chi^2 = 0.54$, P = 0.46). The proportion of crabs with the major chela on the left side of the body reached 46.88%, while 40.63% had it on the right side, although crab handedness was not skewed (Chi-square goodness of fit test, $\chi^2 = 0.14$, P = 0.71) and also not associated to crab sex (Chi-square test for independence, $\chi^2 = 0.08$, P = 0.96). There were four instances (12.5%) of homochely, one female and 3 males; however, individuals in our sample were predominantly heterochelous (Chi-square goodness of fit test, $\chi^2 = 18$, $P < 0.001$).

Blue crab survival

Subjects did not manifest adverse effects of PIT tag implantation and, in general, sedated crabs showed no stress signs as a result of needle insertion through the arthrodial membrane. After needle retrieval, a minute amount of haemolymph leaked through the punctured membrane; however, tags were retained in all individuals and no evident signs of infection or rejection response were identified. The CW of the smallest surviving tagged crabs was 55.26 mm and 57.81 mm for males and females, respectively.

The 77.8% of crabs survived PIT tag implantation without change in behavior. In contrast, mortality reached 22.2% (five males and three females) through the experimental period (Fig. 5). The difference between these two proportions (dif. = 0.56, 95% C.I. = 0.36–0.75) was significant ($Z = 5.67$, $P < 0.001$). The 95% confidence interval of the observed mortality proportion was 0.10–0.39. When the observed mortality proportion was contrasted against the upper bound of its confidence interval, the observed proportion resulted significantly smaller than the hypothesized proportion (Exact method, $P = 0.04$).

Average air temperature during the experimental period of 33 days was 28.32 ± 0.96 °C (26.00–30.20 °C), while the minimum and the maximum reached 21.77 ± 1.54 °C (18.40–25.00 °C) and 34.85 ± 1.42 °C (28.20–37.20 °C), respectively. Continuous temperature readings from inside holding tanks showed crabs were exposed to temperatures over 30 °C for an average of 9 h, daily (Fig. 6). Maximum temperatures were significantly correlated with the percentage of individuals entering status categories leading to death. When the temperature increased, a significantly increasing number of crabs entered the lively to very slow condition. These crabs were previously in very lively condition; however, when the temperature increased their condition deteriorated, as indicated by a negative correlation between the proportion of very lively crabs and increasing temperature ($r = -0.62$; $P = 0.031$; Table 4).

Table 4.

Pearson correlations for maximum temperatures (°C) and condition index categories in captive Cardisoma crassum from Hicaco, Veraguas, Panama.

Condition index	Correlation	95% CI for ρ	P-Value
Dead	0.232	(-0.394, 0.711)	0.468
Very slow	0.737	(0.283, 0.921)	0.006
Slow	0.596	(0.034, 0.872)	0.041
Lively	0.571	(-0.005, 0.862)	0.053
Very lively	-0.622	(-0.881, -0.074)	0.031

Figure 5.

Cardisoma crassum condition and mortality in captivity. Upper panel represents percentage individuals in very lively condition (blank), while the bars bellow contain percentages of lively (diagonal lines), slow (cross), very slow (gray) and dead (black) individuals. Injection day is ID6, and the line in the secondary "y" axis is the maximum temperature °C.

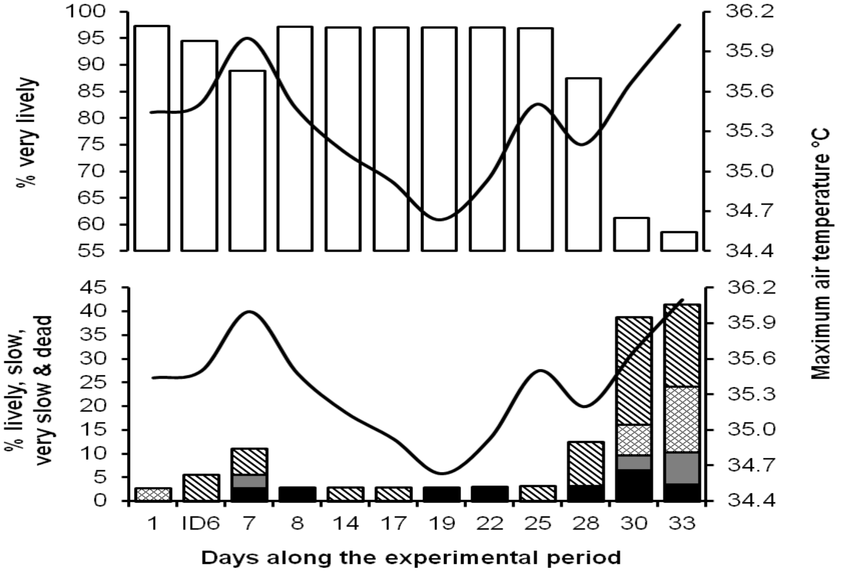
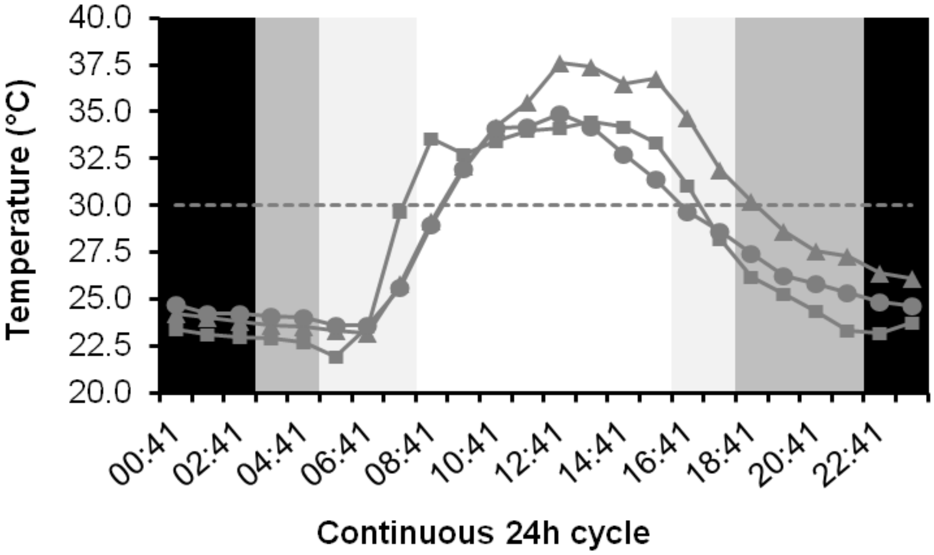


Figure 6.

Temperature from inside Cardisoma crassum holding tanks. Background symbolizes night (black), early morning and evening (gray), morning and late afternoon (light gray), noon and afternoon (blank). Square markers correspond to February records; triangle and circle indicate March and April temperatures, respectively. Dotted line is 30 °C reference.



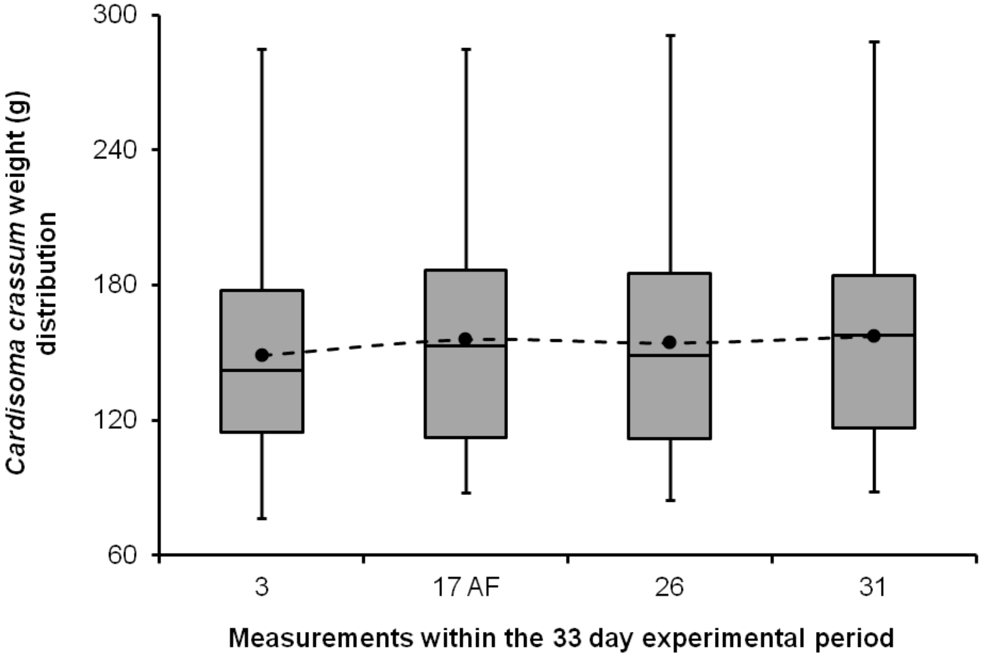
Weight and condition factor response to PIT tag implant

At the start of the experiment, blue crabs weighted in average 148.92 ± 49.51 g (76.41–284.79 g, N = 36), while at the end their average weight was 157.39 ± 51.22 g (88.07–288.03 g, N = 29). Crabs that augmented their weight (81.25 %) had a mean gain of 4.51± 2.91g (0.17–11.79 g, N = 26), while those that lost weight (18.75 %) had an average loss of 4.91 ± 2.65 g (1.78–8.61 g, N = 6).

However, there was no significant difference in mean weight among the four measurements in response to PIT tag implantation (one-way ANOVA, $F_{(3,125)} = 0.17$, $P < 0.913$) (Fig. 7). There was no difference between the sexes (Student t-test, t = -0.33, df = 24, P = 0.74), nor between complete individuals and those missing appendages in weight gains (Student t-test, t = 0.35, df = 24, P = 0.73).

Figure 7.

Captive Cardisoma crassum weight response to PIT tag implantation. Black dots in boxes represent the mean for each weight-in period, and AF is days after tag implant.



There was no difference between condition factor means: Fulton (Student t-test, t = -0.45, df = 64, P = 0.658), Ricker (Student t-test, t = -1.63, df = 63, P = 0.108), and LeCren (Student t-test, t = -0.001, df = 64, P = 0.996) before and after PIT tag implantation (Table 5).

Table 5.

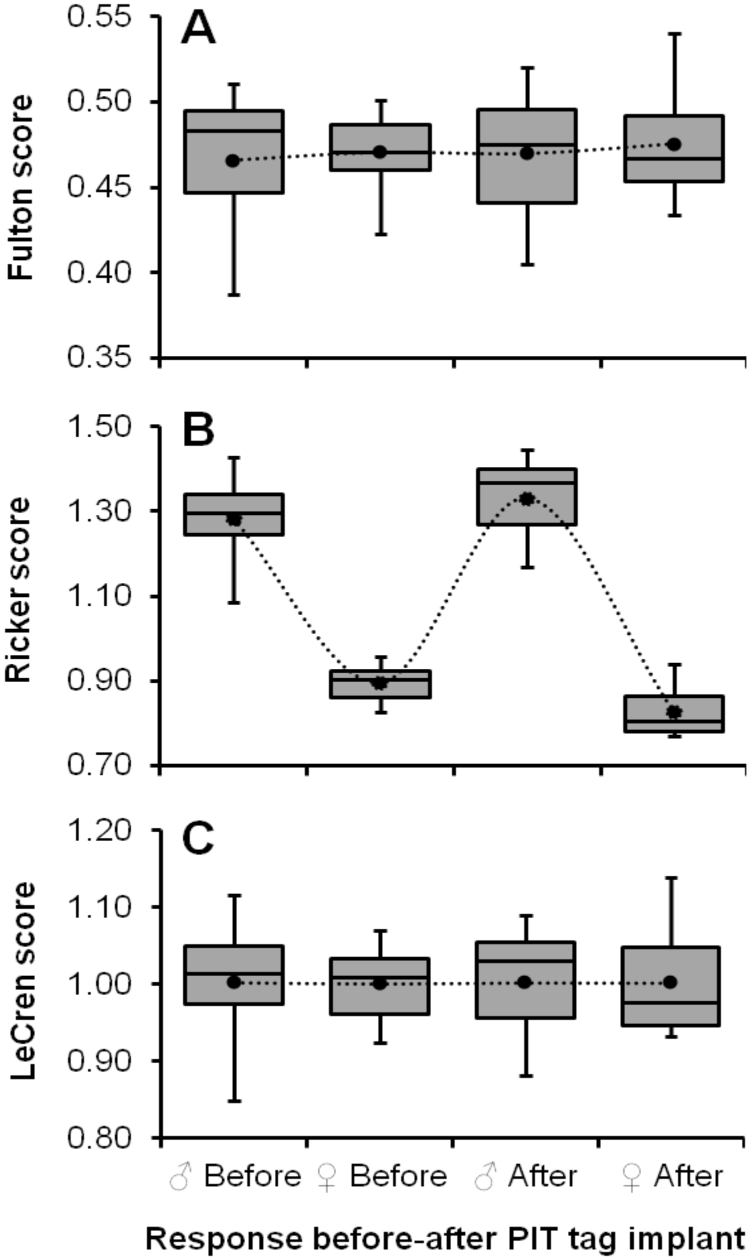
Condition factors before and after PIT tag implant in captive Cardisoma crassum from Hicaco, Veraguas, Panama.

Time	Condition factor	Mean ± SD	Minimum	Maximum
Before PIT tag implant	Fulton's (*k*)	0.467 ± 0.032	0.387	0.511
	Ricker's modified (*k*)	1.167 ± 0.077	0.990	1.295
	LeCren's relative (k_n)	1.002 ± 0.066	0.851	1.112
After PIT tag implant	Fulton's (*k*)	0.471 ± 0.034	0.405	0.540
	Ricker's modified (*k*)	1.198 ± 0.081	1.053	1.364
	LeCren's relative (k_n)	1.002 ± 0.068	0.881	1.141

There was no significant difference between male and female condition factors before PIT tag implantation, except in Ricker's modified condition index (Student t-test, t = 13.03, df = 34, P < 0.001). The same result was obtained while comparing male and female condition factors after PIT tag implantation (Student t-test, t = 18.46, df = 23, P < 0.001; Fig. 8).

Figure 8.

Cardisoma crassum condition factor response before-after PIT tag implantation and individual's sex in captivity. A. Fulton's condition factor. B. Ricker's modified condition factor. C. LeCren's condition factor. Black dots represent condition factor means for each period.



Discussion

This is the first time implantation of PIT tags was attempted successfully in *C. crassum*. Abnormal crab behavior was not observed, and there were no detectable PIT tag effects in weight or any of the three condition factors calculated before and after tag implantation. A difference between sexes was detected through Ricker's condition factor; most likely inherent to body allometry, not condition before and after implantation. In this regard, a small difference in the regression coefficient "b" between sexes could generate large differences in the calculation of this condition factor (Moghaddam et al., 2015). In this study the regression coefficient "b" in females was higher than that of males, causing denominator inflation (Ricker, 1975), which resulted in smaller female condition factor values.

Even though the mortality rate in our experiment reached 22%, the causes for mortality might not have been directly related to the injection or PIT tags themselves. For example, instances of mortality were unlikely due to miscalculation of needle optimal length insertion, as necropsy revealed tags were retained in muscle tissue, at the coxa, without signs of infection or damage to the abdominal cavity. This pattern was observed in individuals that survived to term as well as those of premature death. Haemolymph loss as a result of injection was also an unlikely cause of death, as the amount leaked was negligible among individuals.

Diet was also an unlikely cause for mortality in our experiment since all food items offered were consumed and there was no difference between the four weight-ins. However, we observed differences in appetite within the experimental period, particularly in warm days; feeding activity was low. A portion of individuals seemed to recover but others registered decaying liveliness index scores (Mayze et al., 2014) following such warm days. Moreover, the results point to the importance of high temperatures during the experiment as a factor in the observed mortality proportion.

High temperature and elapsed exposure time (average 9h daily) might have an effect on blue crab dehydration. This is of particular interest for development of aquaculture practices since an increase in dehydration levels leads to a reduction of food intake in Gecarcinid and Fiddler crabs (da Silva et al., 2020; McGaw et al., 2019). Heat stress plays an important role in *Cardisoma crassum* ecology, as in the wild, their habits and fidelity to their burrow may contribute to heat stress avoidance by remaining deep inside burrows, plugging burrow entrances in dry season and becoming active only during cooler night time periods or in rainy season (Lombardo and Rojas, 2022). This pattern is repeated in Fiddler crab species where rising temperature increased the time spent inside burrows (da Silva et al., 2020). Overall, long term heat stress may compromise energy supply, growth, and survival of animals (Pörtner et al., 2017).

Under the current experimental conditions, the mortality proportion would likely remain as low as 10% and under 39% with a 95% confidence level. However, if holding conditions were improved to minimize heat stress, particularly during dry season, lower mortality in research for aquaculture development could be achieved in this species. For instance, PIT tag implantation can be used to advance longer holding times as molting and mating in captivity have not been reported in *C. crassum*. These features of the blue crab's life history are critical to developing breeding programs and determine the potential for aquaculture of the species.

Long term molting in *Cardisoma guanhumi*, a closely related species, can be conductive to internal tag loss (Moraes-Costa and Schwamborn, 2018); thus, longer experimental periods should be tested with PIT tag implanted *C. crassum* individuals, in both captivity and the field, in order to assess long term tag efficiency.

In line with previous studies (Vega et al., 2018; Zambrano y Olivares, 2020), males were larger and heavier than females in our sample. Compared to eastern Montijo Gulf populations studied by Vega et al. (2018) and Lombardo and Rojas (2022), sampled *C. crassum* from the western Montijo Gulf (Hicaco) were larger. Furthermore, we found that the isometric relationship between carapace width (CW) and total weight (TW) in *Cardisoma crassum* was stronger in females, possibly indicating a subtle size difference between the sexes within our sample. This discrepancy could be attributed to variations in the development of body structures. Notably, the female abdomen plays a crucial role in protecting and facilitating egg hatching, leading to changes throughout its allometric development upon reaching sexual maturity (Hartnoll, 1974). The sexual dimorphism observed in the abdomen stems from functional differences in male and female pleopods, resulting in a larger carapace length for female crabs (Hartnoll, 1974; Mclay, 2015). These significant biometric differences may contribute to the more "rounded" cephalothorax observed in female specimens. Consequently, the carapace width and weight increase at almost the same rate, which accounts for the observed CW-TW isometric relationship in females.

Furthermore, we determined a male biased sex ratio; a common condition in crustaceans (Wenner, 1972). Studies published for panamanian populations of *C. crassum* have reported a male biased (Vega et al., 2018) and an even sex ratio (Lombardo and Rojas, 2022). In the case of Hicaco, fishermen indicated that blue crabs are not their main target, a statement confirmed by unavailability of crabs in local markets due to low demand. Normally, fishing mortality eliminates large individuals from the population first; thus, in this case, as there is marked body size asymmetry favoring males, the low fishing mortality (Alemán et al., 2018; Vega et al., 2018; Zambrano y Olivares, 2020), such as in Hicaco, could explain the overall observed size difference and male biased sex ratio in our sample. These arguments should be considered with care, provided size differences as well as the skewed sex ratios might be indicative of population level differences that warrant further research.

Conclusions

Overall, PIT tags appear to be a suitable tagging method for use in *C. crassum*, without negative effects in weight, condition or behavioral features alongside high retention rates and manageable mortality rates.

Acknowledgements

We thank Ciro Lombardo for assistance during the experimental period, and IDEA WILD for partial funding of equipment (Grant ID: LOMBPANA0821). We are also grateful to the reviewers for their valuable comments to improve earlier versions of the manuscript.

References

da Silva, B., Miyai, C. A., Augusto, A., and Costa, T. M. (2020). Effects of temperature increase on the physiology and behavior of fiddler crabs. *Physiology and Behavior*, *215*(December 2019), 112765. https://doi.org/10.1016/j.physbeh.2019.112765

Dinh, H., Coman, G., Hurwood, D. A., and Mather, P. B. (2012). Experimental assessment of the utility of visible implant elastomer tags in a stock improvement programme for giant freshwater prawn (*Macrobrachyum rosenbergii*) in Vietnam. *Aquaculture Research*, *43*(10), 1471–1479. https://doi.org/10.1111/j.1365-2109.2011.02949.x

Dixon, W. J., and Massey, F. J. (1957). Introduction to statistical analysis. In *Introduction to statistical analysis* (2nd ed.). McGraw-Hill.

Drew, M. M., Hartnoll, R. G., and Hansson, B. S. (2012). An improved mark-recapture method using passive integrated transponder (PIT) tags in *Birgus latro* (Linnaeus, 1767) (Decapoda, Anomura). *Crustaceana*, *85*(1), 89–102. https://doi.org/10.1163/156854012X623656

Fischer, W., Krupp, F., Schneider, W., Sommer, C., Carpenter, K. E., and Niem, V. H. (1995). Pacífico centro-oriental. Volumen I. Plantas e invertebrados. In *Guía FAO para la identificación de especies para los fines de la pesca.* (1st ed.). FAO. 646.

Foote, A. R., Stratford, C. N., and Coman, G. J. (2018). Passive integrated transponder (PIT) tagging black tiger shrimp, *Penaeus monodon*: Applications for breeding programs. *Aquaculture*, *491*, 321–324. https://doi.org/10.1016/j.aquaculture.2017.12.029

Forsee, R. A., and Albrecht, M. (2012). Population estimation and site fidelity of the land crab *Cardisoma guanhumi* (Decapoda: Brachyura: Gecarcinidae) on Vieques Island, Puerto Rico. *Journal of Crustacean Biology*, *32*(3), 435–442. https://doi.org/10.1163/193724012X626467

Froese, R. (2006). Cube law, condition factor and weight-length relationships: History, meta-analysis and recommendations. *Journal of Applied Ichthyology*, *22*(4), 241–253.

Fuller, S. A., and McEntire, M. (2013). The Effect of PIT Tagging on Survival, Tag Retention, and Weight Gain in Fingerling White Bass. *Journal of Applied Aquaculture*, *25*(2), 95–101. https://doi.org/10.1080/10454438.2012.759894

Fulton, T. W. (1902). The rate of growth of fishes. *20th Annual Report of the Fishery Board of Scotland*, *3*, 326–446.

Goshima, S., Ono, Y., and Nakasone, Y. (1978). Daily activity and movement of the land crab, *Cardisoma hirtipes* DANA, by radio-telemetry during non-breeding season. *Publications of the Amakusa Marine Biology Laboratory*, *4*(3), 175–187.

Haddaway, N. R., Mortimer, R. J. G., Christmas, M., and Dunn, A. M. (2011). A review of marking techniques for Crustacea and experimental appraisal of electric cauterisation and visible implant elastomer tagging for *Austropotamobius pallipes* and *Pacifastacus leniusculus*. *Freshwater Crayfish*, *18*(1), 55–67. https://doi.org/10.5869/fc.2011.v18.55

Hartnoll, R. G. (1974). Variation in growth pattern between some secondary sexual characters in crabs (Decapoda Brachyura). *Crustaceana*, *27*(2), 131–136.

https://doi.org/https://doi.org/10.1163/156854074X00334

Huxley, J. S. (1950). Relative growth and form transformation. *Proceedings of the Royal Society of London. Series B - Biological Sciences*, *137*(889), 465–469.

Lauzon-Guay, J. S., and Scheibling, R. E. (2008). Evaluation of passive integrated transponder (PIT) tags in studies of sea urchins: Caution advised. *Aquatic Biology*, *2*(2), 105–112. https://doi.org/10.3354/ab00040

Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *British Ecological Society*, *20*(2), 201–219.

Lombardo, R. C., and Rojas, M. (2022). Burrow fidelity in the blue crab, *Cardisoma crassum* Smith, 1870 (Brachyura: Gecarcinidae) from the Ponuga River, Veraguas, Panama. *Nauplius*, *30*, e2022033. https://doi.org/10.1590/2358-2936e2022033

Mayze, J., Poole, S., Mark Boulter, and Iakoba, B. (2014). *Increase sustainable use of crab fisheries resources by recovering revenue from crabs currently rejected at market* (Issue 2012).

McGaw, I. J., Van Leeuwen, T. E., Trehern, R. H., and Bates, A. E. (2019). Changes in precipitation may alter food preference in an ecosystem engineer, the black land crab, *Gecarcinus ruricola*. *PeerJ*, *2019*(5), 1–28. https://doi.org/10.7717/peerj.6818

Mclay, C. (2015). Moulting and growth in Brachyura. In P. Castro, P. Davie, G. D., F. Schram, and C. von Vaupel (Eds.), *Treatise on Zoology - Anatomy, Taxonomy, Biology. The Crustacea* (pp. 245–316). Brill. https://doi.org/10.1163/9789004190832_007

Meynecke, J. O., Mayze, J., and Alberts-Hubatsch, H. (2015). Performance and physiological responses of combined t-bar and PIT tagged giant mud crabs (*Scylla serrata*). *Fisheries Research*, *170*, 212–216. https://doi.org/10.1016/j.fishres.2015.06.013

Moghaddam, P., Kalvani Neitali, B., Kamrani, E., Noori, A., Pinheiro, M. A. A., and Akbarzadeh, A. (2015). Condition factor and carapace width versus wet weight relationship in the blue swimming crab *Portunus segnis*. *Animal Biology*, *65*(2), 87–99. https://doi.org/10.1163/15707563-00002463

Moraes-Costa, D., and Schwamborn, R. (2018). Site fidelity and population structure of blue land crabs (*Cardisoma guanhumi* Latreille, 1825) in a restricted-access mangrove area, analyzed using PIT tags. *Helgoland Marine Research*, *72*(1), 1–15.

Ogle, D. (2016). *Introductory Fisheries Analyses with R* (Taylor and Francis Group (ed.)). Chapman and Hall/CRC.

Ogle, D., Doll, J., Wheeler, P., and Dinno, A. (2021). *FSA: Fisheries Stock Analysis* (0.9.1.9000).

Pörtner, H. O., Bock, C., and Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: Bridging ecology and physiology. *Journal of Experimental Biology*, *220*(15), 2685–2696. https://doi.org/10.1242/jeb.134585

Rasal, A., Patnaik, M., Murmu, K., Sundaray, J., and Das Mahapatra, K. (2021, December). Importance of PIT Tagging in Aquaculture Selective Breeding Programs. *Vol. 52 No. 4*, *December*, 59–63.

Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada*, *191*, 1–382.

Rondeau, A., and Sainte-Marie, B. (2016). Variable mate-guarding time and sperm allocation by male Snow Crabs (*Chionoecetes opilio*) in response to sexual competition, and their impact on the mating success of females. *Biological Bulletin*, *201*(2), 204–217.

Sato, T., Sugaya, T., and Yoshikawa, H. (2020). Novel method of tagging the kuruma prawn *Penaeus japonicus* with a trans-molting retentive external eye (TRAMORE) tag. *Fisheries Research*, *225*, 105482. https://doi.org/10.1016/j.fishres.2019.105482

Sato, T., Yoseda, K., Abe, O., Shibuno, T., Takada, Y., Dan, S., and Hamasaki, K. (2013). Growth of the coconut crab *Birgus latro* estimated from mark-recapture using passive integrated transponder (PIT) tags. *Aquatic Biology*, *19*(2), 143–152. https://doi.org/10.3354/ab00517

Schwamborn, R., and Moraes-Costa, D. F. (2021). Growth and mortality of the endangered land crab *Cardisoma guanhumi* assessed through tagging with PITs and novel bootstrapped methods. *Pan-American Journal of Aquatic Sciences*, *16*(1), 57–78.

Sharp, W. C., Lellis, W. a, Butler, M. J., Herrnkind, W. F., Hunt, J. H., Pardee-woodring, M., and Matthews, T. R. (2000). The use of coded microwire tags in mark-recapture studies of juvenile Caribbean Spiny Lobster, *Panulirus argus*. *Journal of Crustacean Biology*, *20*(3), 510–521.

Skurdal, J., Qvenild, T., and Taugbol, T. (1992). Mark-recapture experiments with noble crayfish, *Astacus astacus* L., in a Norwegian lake. *Aquaculture and Fisheries Management*, *23*(2), 227–233.

Smith, I. P., Jensen, A. C., Collins, K. J., and Mattey, E. L. (2001). Movement of wild European lobsters *Homarus gammarus* in natural habitat. *Marine Ecology Progress Series*, *222*, 177–186. https://doi.org/10.3354/meps222177

Ulmestrand, M., and Eggert, H. (2001). Growth of Norway lobster, *Nephrops norvegicus* (Linnaeus 1758), in the Skagerrak, estimated from tagging experiments and length frequency data. *ICES Journal of Marine Science*, *58*(6), 1326–1334. https://doi.org/10.1006/jmsc.2001.1133

Vega, Á. J., Mena, C., y Robles, Y. (2018). Pesca artesanal de *Cardisoma crassum* (Cangrejo) y *Ucides occidentalis* (Mangote) en el sector nororiental del Golfo de Montijo, Pacifico de Panamá. *Tecnociencia*, *20*(2), 5–22.

Wenner, A. M. (1972). Sex ratio as a function of size in marine Crustacea. *The American Naturalist*, *106*(949), 321–350.

Zambrano, R., y Olivares, S. (2020). Alometría y madurez sexual morfométrica de *Cardisoma crassum* (Decapoda: Gecarcinidae) en la costa continental norte de Ecuador. *Geomare Zoologica*, *2*(2), 25–33.