

## Combined use of artificial intelligence and biological oscillatory growth model to explain the relationship: shell length vs. dye production in purple snail

## Uso combinado de inteligencia artificial y un modelo oscilatorio de crecimiento para explicar la relación: longitud de la concha vs. producción de tinte del caracol púrpura

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### ABSTRACT

**Background.** The purple snail is ancestrally important along the coast of Oaxaca as its dye is used by the indigenous Mixtec people to dye culturally valuable cotton yarns. A snail population located in a protected area named Huatulco National Park (HNP) is decreasing because specimens of all sizes are targeted more than once for dye extraction throughout the exploitation season (from October to March). This cultural practice occurs because the indigenous Mixtec people believe that dye production is continuous and increases proportionally in relation to shell length. **Goals.** The objective of this study was to establish the relationship between the total volume of extracted dye and the total length of the shell. **Methods.** In this study we implemented a neural regression model with artificial intelligence and a biological oscillatory growth model to demonstrate that dye production does not increase proportionally in relation to shell length. **Results.** The results suggest that snails of all sizes require a break to synthesize more dye after milking. Smaller snails of 2.7 to 4.2 cm produce a small volume of dye and conduct energy to growth, while larger snails of 4.8 to 7.7 cm produce a large volume of dye, but grow less. **Conclusion.** Our results allow us to conclude that dye production does not increase proportionally in relation to changes in shell length. At any size, a snail requires a rest to synthesize more dye after being milked. Special marks should be used to identify milked specimens in order to avoid repetitive dye extraction.

**Key words:** Dye production, cultural value, Huatulco, resources management, purple dye snail.

### RESUMEN

**Antecedentes.** El caracol púrpura ha sido ancestralmente importante a lo largo de la costa de Oaxaca ya que su tinte es utilizado por los mixtecos para teñir hilos de algodón. En el área protegida Parque Nacional Huatulco (PNH) la población de caracol púrpura está disminuyendo debido a que a los especímenes de todos los tamaños, se les extrae repetidamente su tinte durante la temporada de explotación (de octubre a marzo). Esta práctica ocurre porque los mixtecos creen que la producción de tinte es continua y que aumenta proporcionalmente con la longitud de la concha. **Objetivos.** El objetivo de este estudio fue establecer la relación entre el volumen total del tinte extraído y la longitud total de la concha del caracol púrpura. **Métodos.** Implementamos un modelo de regresión neuronal con inteligencia artificial y un modelo oscilatorio de crecimiento biológico para demostrar el tipo de relación entre la producción de tinte y la longitud de la concha. **Resultados.** Los resultados sugieren que los caracoles de todos los tamaños requieren un descanso para sintetizar más tinte después de la ordeña. Los caracoles más pequeños de 2.7 a 4.2 cm producen un pequeño volumen de tinte y conducen la energía al crecimiento, mientras que los caracoles más grandes de 4.8 a 7.7 cm producen un gran volumen de tinte, pero crecen menos. **Conclusión.** La producción de tinte no aumenta proporcionalmente con la longitud de la concha. A cualquier tamaño, un caracol requiere un descanso para sintetizar más tinte después de ser ordeñado. Se deben usar marcas especiales para identificar los especímenes ordeñados a fin de evitar la extracción repetitiva de tintes.

**Palabras clave:** Producción de tinte, valor cultural, Huatulco, manejo de recursos, caracol tinte púrpura.

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## INTRODUCTION

The snail *Plicopurpura pansa* Gould, 1853 is geographically distributed along the coasts of the Tropical Pacific Ocean from Baja California, Mexico, to Peru and the Galapagos Islands (Clench, 1947; Peña, 1970; Paredes *et al.*, 1999). Its habitat is the rocky intertidal fringe for the entirety of its lifespan (García-Ibáñez *et al.*, 2007; Chávez & Michel-Morfin, 2006; Naegel & García-Domínguez, 2006).

In the Huatulco National Park (HNP), *P. pansa* is known as the Purple Dye Snail because it synthesizes and exudes a colored secretion called "purple dye" that the species uses to hunt and to protect itself from environmental stress and predation (Schunck, 1880; Garrity, 1984; Michel-Morfin *et al.*, 2000; Naegel & Cooksey, 2002). It is considered an ancestrally important inhabitant because its dye is used by the Mixtec people of Oaxaca to dye cotton yarns for culturally valuable ceremonial clothing (Ramírez-Rodríguez & Naegel, 2003). The exploitation season of *P. pansa* in the HNP is from October to March (Turok *et al.*, 1988). The Mixtec people have asserted that specimens of *P. pansa* do not die when their dye is extracted and, according to them, the exploitation of the snails in the HNP has remained sustainable since the 1980s. However, Cervantes-Hernández *et al.* (2016) documented that the *P. pansa* population in the HNP has been over-exploited since 1980, and the main problem of this over-exploitation is the disordered extraction of dye that the Mixtec people have carried out on all snail sizes.

Previous studies include the synthesis and chemical composition of the dye by Baker & Duke (1973), Verheeken (1989), Naegel & Cooksey (2002) and Ngangbam *et al.* (2019), the age-length relationship of *P. pansa* by Cervantes-Hernández *et al.* (2010) and the relationship between dye production and shell length changes by Turok *et al.* (1988) and Michel-Morfin *et al.* (2002). On the other hand, laboratory studies have shown that after the extraction of dye, adults of *P. pansa* require

time and energy to synthesize more dye (Naegel, 2005; Naegel & López-Rocha, 2006). In contrast with those laboratory results, Turok *et al.* (1988) and Michel-Morfin *et al.* (2002) suggested that dye production increases proportionally in relation to changes in shell length.

Notwithstanding that exploitation of *P. pansa* continues in the HNP, the scientific sampling of specimens has been completely prohibited. The last snail sampling that took place in the HNP was carried out by UMAR (Universidad del Mar) personnel from 2011 to 2012. The data on total volume of extracted dye and total shell length generated by the UMAR personnel were used in the present study to implement a neural regression model with artificial intelligence in order to find the best trend line (Haykin, 1994), and to graphically fit the  $V_t$ - $L_t$  variation. This was then used to explain how dye production relates to changes in shell length. We proposed a biological growth model to replicate the trend line found in the neuronal process, and to generate predictions of  $V_t$  for a variety of  $L_t$  data recorded for *P. pansa* specimens.

## MATERIALS AND METHODS

### Study area

Huatulco is the most important tourist destination on the central coast of the state of Oaxaca, Mexico ( $15^{\circ}39'08.98''$ – $15^{\circ}47'07.76''$  N and  $96^{\circ}06'26.18''$ – $96^{\circ}14'14.29''$  W). The HNP was decreed a natural protected area in 1998, including the coastal ecosystems located from San Agustín Bay to Violin Bay (CONANP, 2003; SEMARNAT, 2008) (Fig. 1). The fauna that inhabits the HNP includes 291 bird species, 166 of fish, 130 of mammals, 72 of reptiles and 70 of invertebrates (including 12 coral species) (Cervantes-Hernández *et al.*, 2016). The exploitation of *P. pansa*, artisanal fisheries and tourism are the main economic activities that take place in the HNP (Cervantes-Hernández & Egremy-Valdez, 2013; Cerdanars *et al.*, 2014; Alejo-Plata *et al.*, 2019).

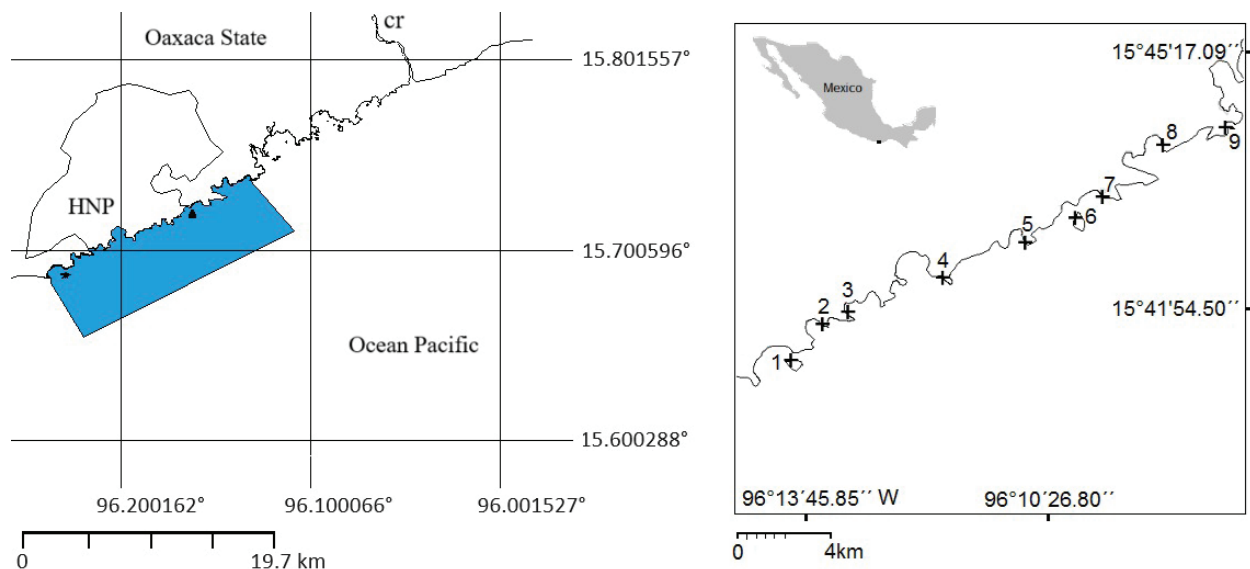


Figure 1. Geographical location of Huatulco tourist destination and the protected coastal ecosystems in Huatulco National Park (HNP) (blue polygon), St. Agustín Island (1), Riscalillo Bay (2), Jicaral Bay (3), La India Bay (4), Palo Santo Bay (5), Cacaluta Island (6), Cacaluta Bay (7), El Organo Bay (8) and Violin Bay (9). Copalita River (CR).

## Fieldwork

Two samplings were carried out in coastal ecosystems of the HNP with a low touristic access, at St. Agustín Island, Riscalillo Bay, Jicaral Bay, La India Bay, Palo Santo Bay, Cacaluta Island, Cacaluta Bay, El Organo Bay and Violin Bay (Fig. 1). The first sampling took place from September to November 2011 during the first week of each month, and the second sampling was carried out exclusively in St. Agustín Island during two weeks of June 2012. Fieldwork was done using a 6.4 cm thick and 50 m long polypropylene rope deployed parallel to the coastline along the rocky intertidal fringe, where snails were randomly sampled 1 m to the left and 1 m to the right of the rope, in a total area of 100 m<sup>2</sup>. As the Mixtec people usually do, each snail was carefully removed from a rocky crevice and a moderate pressure was applied to its operculum to extract the dye. The dye was put into a plastic jar to measure its volume.

A total of 252 specimens were sampled (99 males and 153 females). The total length of the shell was measured as is shown in Figure 2, (Cervantes-Hernández *et al.*, 2010; 2016). Males were distinguished from females by the presence of copulatory organs (penises) (Turok *et al.*, 1988). The total volume of extracted dye (Vt, mL) and the total length of each shell (Lt, cm) were recorded.

From literature it is known that specimens greater than 1.8 cm are sexually mature and younger recruits are from 1.0 to 1.8 cm (Naegel & García-Domínguez, 2006; Cervantes-Hernández *et al.*, 2016). Younger recruits produce very little dye, and they are more vulnerable to dye extraction (Castillo-Rodríguez & Amezcua-Linares, 1992). For this reason, these small specimens were not considered in the present study. To avoid the presence of younger recruits in our samples, the two samplings were specifically conducted during reproductive season of *P. pansa*. Cervantes-Hernández *et al.* (2016) reported that complete reproductive season spanned from March to October/November, with the highest positive cyclical anomaly signal during June/July. Thus, all specimens sampled in HNP were sexually mature greater than 2.7 cm.

## Dye production-shell length relationship

Once the data were recorded, a Vt-Lt dispersion graph was plotted. We then designed a neural regression model with artificial intelligence in order to find the best trend line (Haykin, 1994) and graphically fit the Vt-Lt variation. During this neuronal process, artificial intelligence executed many linear and non-linear model combinations to find the

best trend line, and once this last was concluded, in its final training, the parameters of these models were estimated following minimum squares in the Statistica software version 7.0.

## Biological growth model implementation

The regression neuronal model cannot be used to generate predictions of Vt because biological growth parameters are not included in its mathematical structure (e.g. the asymptotic length ( $L_{\infty}$ ), the metabolic growth ( $k$ ), the age at zero cm ( $t_0$ )). For this reason, we reviewed the biological oscillatory growth models documented in the literature to find one that could approximately replicate the trend line found in the neuronal process. We specifically focused on biological oscillatory growth model of Pitcher and MacDonald (1973) to explain size changes in short time intervals conditioned by biological requirements (e.g. synthesizing a secondary metabolite such as the *P. pansa* dye), and because this model includes biological growth parameters such as those mentioned above.

As the biological oscillatory growth model of Pitcher and MacDonald (1973) explains the age-length relationship (At-Lt), we modified the mathematical structure, replacing the  $L_{\infty}$  variable (the asymptotic length) by the  $V_{\infty}$  variable (the maximum dye volume that a male or female snail can theoretically produce throughout its life), and the Et variable by the Lt variable. Our mathematical proposal is as follows,

$$V_t = V_{\infty} \cdot (1 - \exp(-q)) \quad (1)$$

with

$$q = C \cdot [(\sin(2\pi(L_t - L_s)) / LA) + k(L_t - L_m)]$$

Where  $V_{\infty}$  is in mL,  $C$  parameter controls the amplitude of oscillations in the sine function,  $L_s$  parameter defines where each oscillation in the sine function begin,  $LA$  is average length change observed in the records of Lt,  $k$  parameter is the monthly metabolic growth rate of snail shell length and  $L_m$  parameter is the minimal length recorded in a snail specimen in HNP (2.7 cm). The parameters set included in Eq. 1 were estimated with the Bayesian algorithm of likelihood (Haddon, 2011), considering that estimation error ( $\epsilon = V_t \text{ observed} - V_t \text{ expected}$ ) has a log-normal distribution:

$$-\ln \ell(V_{\infty}, C, L_s, LA, k / V_t, L_t) = \sum \ln(DE\epsilon) + \ln(2\pi)/2 + \epsilon^2/(2 DE\epsilon^2) \quad (2)$$

Where  $DE\epsilon = \sqrt{(1/n) \cdot \sum \epsilon^2}$  with  $n = 252$  specimens sampled. The Eqs. 1 and 2 were solved in the Excel software version 2007.

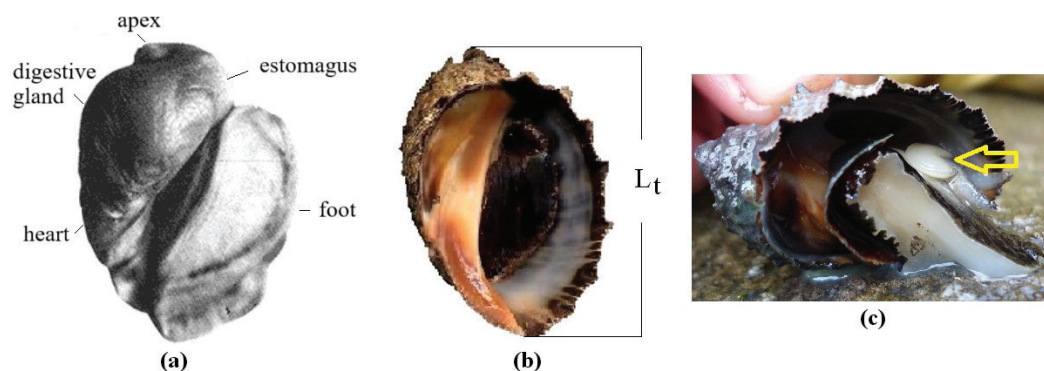


Figure 2. Ventral view without shell of *P. pansa* (a) (Castillo-Rodríguez and Amezcua-Linares, 1992), ventral view of the shell (b) and the yellow arrow shows the copulatory organ (penis) in a male specimen (c).

The oscillatory growth model of Pitcher and MacDonald (1973) was structured with male and female information, and it was not focused to particular sex. For this reason, the results of oscillatory growth model describe how a male or female snail produce dye as it grows.

The statistical comparison between trend lines generated with regression neuronal model (observed fit) and Pitcher and MacDonald's model (expected fit) were evaluated with  $\chi^2$  distribution (Zar, 1999).

## RESULTS

### Dye production-shell length relationship

The regression neuronal model was structured with three learning layers (Fig. 3), where the input data are the records of Lt (independent variable) and the output data are expected Vt values (dependent variable). The first learning layer is composed of one neuron, the second learning layer is composed of nine neurons, and the third learning layer is composed of one neuron.

The neuron in the first learning layer was activated by a simple linear model [ $a_0 = (0.19 \cdot Lt) - 0.52$ ]. Coming out from  $a_0$ , the following nine synaptic signals were structured [ $b_1 = (a_0 - 0.33)^2 \cdot 13.14$ ,  $b_2 = (a_0 - 0.80)^2 \cdot 2.69$ ,  $b_3 = (a_0 - 0.05)^2 \cdot 5.06$ ,  $b_4 = (a_0 - 0.97)^2 \cdot 1.51$ ,  $b_5 = (a_0 - 0.60)^2 \cdot 5.26$ ,  $b_6 = (a_0 - 0.13)^2 \cdot 8.07$ ,  $b_7 = (a_0 - 0.18)^2 \cdot 10.59$ ,  $b_8 = (a_0 - 0.24)^2 \cdot 13.28$ ,  $b_9 = (a_0 - 0.44)^2 \cdot 8.09$ ]. An exponential model (E) was used to activate the nine neurons in the second learning layer [ $c_1 = E^{-b_1}$ ,  $c_2 = E^{-b_2}$ ,  $c_3 = E^{-b_3}$ ,  $c_4 = E^{-b_4}$ ,  $c_5 = E^{-b_5}$ ,  $c_6 = E^{-b_6}$ ,  $c_7 = E^{-b_7}$ ,  $c_8 = E^{-b_8}$ ,  $c_9 = E^{-b_9}$ ]. Coming out from each active neuron in the second learning layer, nine linear models were combined to structure the following single synaptic signal [ $d_0 = [(c_1 \cdot 67.03) + (c_2 \cdot -276.87) + (c_3 \cdot 790.34) + (c_4 \cdot 506.45) + (c_5 \cdot 120.64) + (c_6 \cdot 1177.12) + (c_7 \cdot 803.99) + (c_8 \cdot -239.03) + (c_9 \cdot -67.12)] - 308.37$ ]. Finally, the neuron in the third learning layer was activated by a simple linear model, and this includes a statistic safety threshold [ $e_0 = |(d_0 \cdot 0.08) - 0.28| - 0.5$ ], where  $e_0$  are the ex-

pected Vt values. The regression neuronal model recorded a  $R^2$  value of 0.98.

An ascendant ladder fit consisting of four echelons was generated by artificial intelligence (Fig. 4), and reached a maximum dye production of 10.9 mL at 7.0 cm. The formation of lines with smooth and abrupt slopes was observed between one echelon and the next, suggesting that when dye production is slow, size increases (smooth slope), and when dye production is fast, size increases more slowly (abrupt slope). The presence of echelons in the regression neuronal model suggests that this species presents rests in dye production during its lifespan, and apparently it is during these rests that size increases are more noticeable.

Based on these data, we suggest that dye production does not increase proportionally in relation to changes in shell length. The population of *P. pansa* included mainly small (2.7 cm) and large (7.7 cm) females, with males smaller than 4.7 cm recorded producing low volumes of dye. So, small snails (2.7–4.2 cm) produce small dye volumes while growing faster, whereas larger snails (4.8–7.7 cm) produce greater dye volumes while growing slower.

### Biological growth model implementation

The solved oscillatory growth model of Pitcher and MacDonald (1973) is shown in Eq. 3 and Figure 5.

$$Vt = 10.9 \cdot (1 - \exp(-q)) \quad (3)$$

With:

$$q = 0.023 \cdot [(\sin(2\pi(Lt - (-0.005)))) / 0.8] + 0.16(Lt - 2.7)$$

In concordance with Eq. 3 a female snail can produce a maximum 10.9 mL when reach an age greater than 7.0 cm. This Pitcher and MacDonald's model recorded a  $R^2$  value of 0.96.

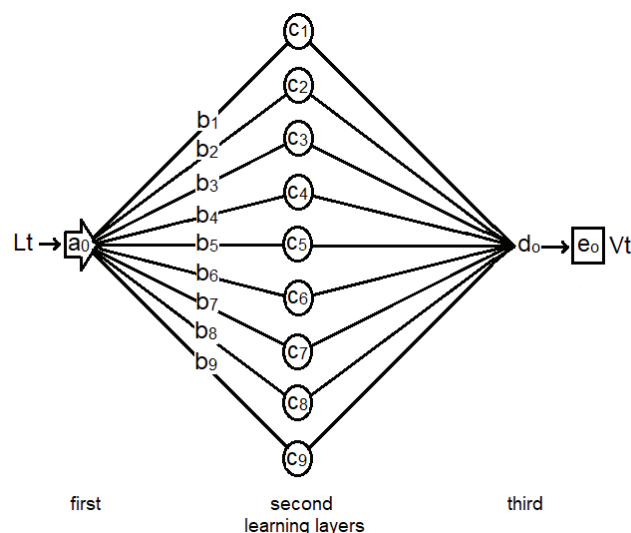


Figure 3. Structure of regression neuronal model generated with artificial intelligence, total volume of extracted dye (Vt), total length of the shell (Lt).

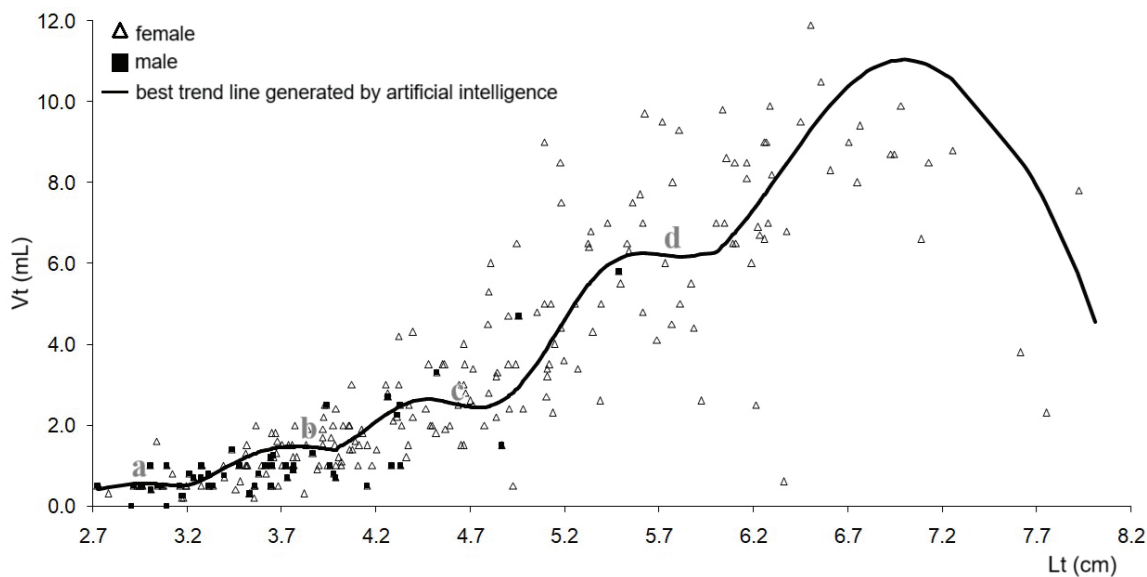


Figure 4. The fitted variation  $V_t$ - $L_t$  with the regression neuronal model (black line), total volume of extracted dye ( $V_t$ ), total length of the shell ( $L_t$ ). First (a), second (b), three (c) and fourth (d) echelon.

The statistical comparison between trend lines generated with regression neuronal model (observed fit) and our Pitcher and MacDonald's model (expected fit) were not significant ( $\chi^2 = 50$ ,  $\chi^2_{0.05} = 289$ , degrees of freedom = 251,  $p > 0.05$ ). Thus, our mathematical proposal of Pitcher and MacDonald should offer reliable predictions of  $V_t$  for a variety of records  $L_t$  in *P. pansa* specimens.

## DISCUSSION

Cervantes-Hernández *et al.* (2010; 2016) documented that the population of *P. pansa* has been overexploited in the PNH since 1980, resulting in a modification of the age structure and length ranges, with a general tendency to equalize sizes in both sexes. Authors mentioned that in HNP male specimens are becoming increasingly scarce and female specimens are increasing. Our observations are consistent with the reported by these authors, because our snail sample was strong represented by female specimens in comparison to male specimens.

Unlike Turok *et al.* (1988) and Michel-Morfin *et al.* (2002), our results indicate that dye production does not follow a continuous increasing relationship with shell length. In the present study, the dye production-shell length relationship was better explained by a pattern similar to an ascendant ladder with four echelons (Fig. 4), where each echelon indicated a break in dye production.

As is the case of bacterial growth, secondary metabolites do not form part of the biomass (Varela & Grotiuz, 2006), and its synthesis, as was documented in this study on the production of dye, occurs in the sections where growth in size and biomass slow down (e.g., in the linear sections with abrupt slopes indicated in Figure 4). The formation of the echelons in Figure 4 indicates pauses in the synthesis of the dye, as García *et al.* (2007) documented for secondary metabolites of bacteria, during stationary phases, when bacterial growth is activated.

In a previous study carried out in the HNP by Turok *et al.* (1988), the dye production-shell length relationship was reported only with a curvilinear fit, with no mathematical equation or statistic correlation value. We clearly see here that the reported fit is non-linear and includes an ascendant ladder fit consisting of two echelons reaching a maximum dye production of 5.5 mL at 8.0 cm. Turok *et al.* (1988) attributes the inflection points to the use of male and female data in conjunction, highlighting that dye production stopped and size increased at their inflections.

Michel-Morfin *et al.* (2002) reported a potential fit with a maximum dye production of 6 mL at 5.0 cm ( $V_t = 6E^{-6} \cdot L_t^{3.41}$ ) using only nine data recorded for both sexes along the Mexican Pacific between the southern coast of Baja California and Socorro Island in the state of Jalisco (with an estimated statistical correlation value of 0.81,  $p < 0.05$ ). From a visual examination of their Fig. 4 graph with a potential fit, the observed data suggest a low dye production by fast growing small specimens (potential echelon) between 2.0 and 3.0 cm, where small specimens slowly produced low dye volumes and grew faster. No other echelons were identified since very few specimens larger than 3.0 cm were sampled by these authors.

When Turok *et al.* (1988) and Michel-Morfin *et al.* (2002) established their *P. pansa*  $V_t$ - $L_t$  relationship, they did not consider, as Naegel (2005) and Naegel & López-Rocha (2006) had mentioned, that after the extraction of dye the specimens require time and energy to synthesize more dye. Thus, the first authors explained this particular relationship without considering any biological parameter in their proposed mathematical equations.

In comparison with modern biological growth models (Zimmer *et al.*, 2014), Pitcher and MacDonald (1973) did not include biological parameters such as age of maturity (energy invested in sexual maturation), the reproduction buffer (energy that is available to invest in

reproduction), food limitation (when the available food is not sufficient or suitable for growth) and the effect of temperature. Despite the deficiencies mentioned, we consider that Pitcher and MacDonald's model is an alternative to generate predictions of  $V_t$ , as the regression neuronal model generated a preliminary fit with sufficient biological variation to establish that dye production does not increase with a fixed rate in relation to changes in shell length. The derivation of Pitcher and MacDonald's model was explained by Somers (1988), and this derivation is similar to replacing the  $L_{\infty}$  variable by the  $V_{\infty}$  variable and the  $E_t$  variable by the  $L_t$  variable.

Cervantes-Hernández *et al.* (2016) documented that the *P. pansa* population in the HNP has been over-exploited since 1980, and stated that the main cause of this was that the Mixtec people extract dye from specimens of all sizes, as well as repeatedly from one same specimen. Turok *et al.* 1988 and Michel-Morfin & Chávez (2000) indicated that a rest of 20 to 21 days should be established between extractions in order to end repetitive dye extraction and increase the abundance of snails in the HNP. Naegel (2005) in turn mentioned that this species cannot produce new dye during the 24 hours following extraction. Despite the recommendations, the extraction practice has not respected any suggestion or indication over the last 22 years and the population of snails continues to be over-exploited (Cervantes-Hernández *et al.*, 2016). Repetitive dye extractions take place as various indigenous groups follow the same route along the rocky shores (on a round trip). Consequently, snails die from over-handling although the Mixtec people say that they remove them carefully.

Naegel (2005) mentioned that dye extraction at any age of *P. pansa* affects the production of mucus that facilitates adhesion and movement along the rocky intertidal fringe, thus handling may affect the snails' adhesion to rocks (Naegel & López-Rocha, 2006). Despite the Mixtecs

manipulating the snails during low tides, the adverse effects of dye extraction can persist until the time of the next high tide causing a high mortality. Thus, much handling has negative effects on *P. pansa* through stress, a greater predation (Garritty, 1984) and a decreased capacity to adhere to rocks.

## CONCLUSIONS

The population of *P. pansa* in the HNP consists mainly of females producing large dye volumes, whereas male production is scarce. Dye production does not increase proportionally in relation to changes in shell length. At any size, a snail requires a rest to synthesize more dye after being milked. Small 2.7 to 4.2 cm snails produce a small volume of dye with big length increases, whereas larger 4.8 to 7.7 cm snails produce a large volume of dye with small length increases. Special marks should be used to identify milked specimens in order to avoid repetitive dye extraction.

## Etic and contribution statement

Pedro Cervantes-Hernández: Conceptualization, Formal analysis, Project administration, Resources, Writing – original draft, Writing – review & editing. Ana L. Ibañez: Project administration, Writing – review & editing. Ana Maria Torres-Huerta: Project administration, Writing – review & editing.

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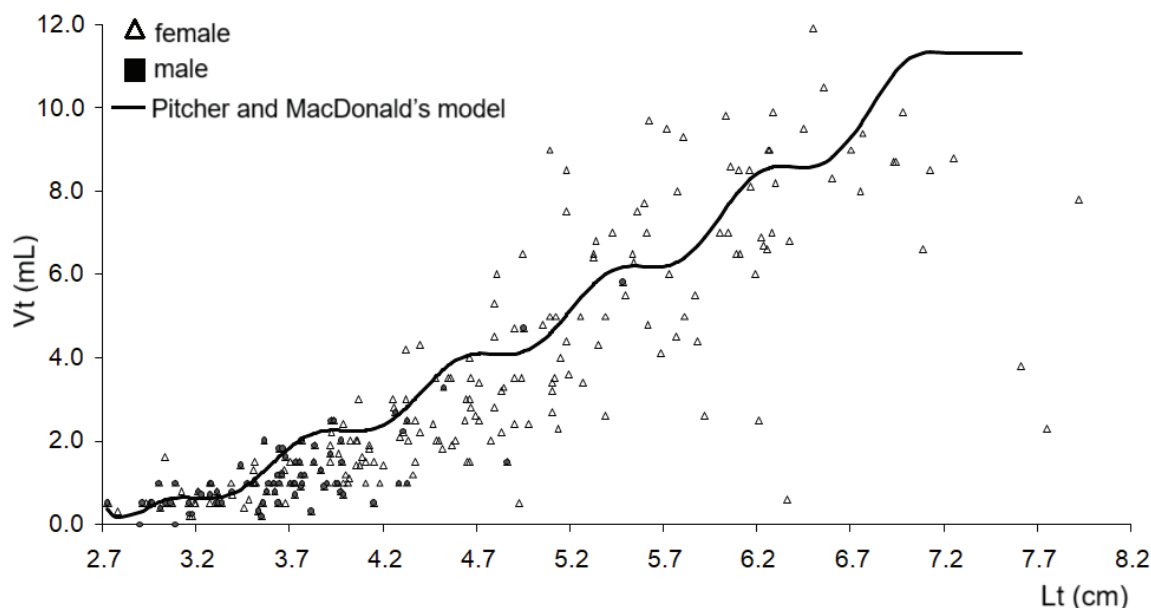


Figure 5. The fitted variation  $V_t$ - $L_t$  with the Pitcher and MacDonald's model (black line), total volume of extracted dye ( $V_t$ ), total length of the shell ( $L_t$ ).

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