Estimation of the Abundance and Population Structure of the Purple Snail *Plicopurpura pansa* (Gould, 1853) Comparing Two Methods

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Abstract: A study using a mark-recapture method was used in order to estimate the abundance and population structure of the Purple snail *Plicopurpura pansa* (Gould, 1853), on a rocky shore in Tenacatita Bay, Jalisco, Mexico. A comparison of the results obtained by this method and the linear transects parallel to coast was performed, the latter has been the method most commonly used in previous studies for this species. The results indicate significant differences in estimates of population parameters obtained by the two methods used; we found that in a general of linear transection methods tend to underestimate the abundance of these snails. Given the implications of these findings for the management of a species with potential for exploitation, such as the Purple snail, we discuss the possible causes and significance of this discrepancy of the methods.

INTRODUCTION

Among the marine mollusks that produce dye, the most important is the purple snail or purple conch *Plicopupura pansa* (Gould 1853, synonymous: *Purpura pansa)*, which has been used by some Indigenous people in America since two thousand years ago to obtain a dye in purple color [1]. Nowadays, The Mixtecos from the Mexican Pacific coast use the dye secreted by *P. pansa,* combining it with other natural inks such as cochineal carmine, from the pearl cactus insect *Dactylopius coccus*, and indigo, from plants of the genus Indigofera, in order to dye ceremonial dresses [2].

The purple snail is distributed in the East Tropical Pacific along the Panamic province, from Baja California, Mexico, up to the south of Colombia, including the Coco, Malpelo and Galapagos Islands [3, 4]. It is often found attached to the wave exposed to rocky substrata in the intertidal zone, building up a horizontal fringe parallel to the coast line. They are active predators feeding themselves from other invertebrates.

The most important characteristic and the fact that makes this snail different from others that also produce (species of *Murex*, *Purpura* and *Thais*), is that it is not necessary to break its shell to obtain the dye. In fact, if the process is done in a correct way, it is possible to obtain successive milking without affecting its population [5]. Therefore, *P. pansa* is a species that, due to its characteristics, represents an opportunity to take advantage of the ink it produces [6]. However, before it could be used it is very important to count with an appropriate estimate of its population size.

The linear transects has been the most commonly used method in previous studies among the different groups of investigators, to determine the relative density of the species [1, 7-12], however, some other different methods have also been applied [13-18]. There is a possibility that some of the organisms stayed out of our reach while we made our sample, therefore this method might sub estimate the abundant population. This maybe caused, among other things, by the changes of the sea level or the snails movement due to their reproduction and feeding.

It was considered that by developing a mark and recapture experiment, more trusting data of the estimate size of the population would be obtained. Using a method in which the size of the population would be determined in consecutive moments it could be less sensible to the space distribution changes of the species or the vulnerability of the snail from being collected. This would provide information about the differences in the estimated density between both methods and as a result, an estimation of the efficiency of the most commonly used method until now.

MATERIALS AND METHODOLOGY

The study took place at a rocky shore at Tenacatita´s bay, Jalisco's South Coast in Mexico. A portion of the rocky beach was chosen locally known as "Las Coloradas" (Fig. **1**). By previous samples it is known that it is an area where the purple snail is abundant. Due to its difficult access it is considered that the anthropogenic disturbances are low or even none. The georeferenced location of the place is 19°17.516' L.N. y 104°50.329'L.W.

Since we sought to comply with the assumptions of closed population necessary for the method of capture and recapture, a limited rocky zone was chosen having both sides small sand beaches and rocks. These characteristics were needed since it has been observed that in these places the purple snail are limited, and the natural barrier limits them from moving to a different place. For the labeling period the following procedure was done. First, a permanent color paint label was put on the rocks at a distance of 25 meters along a

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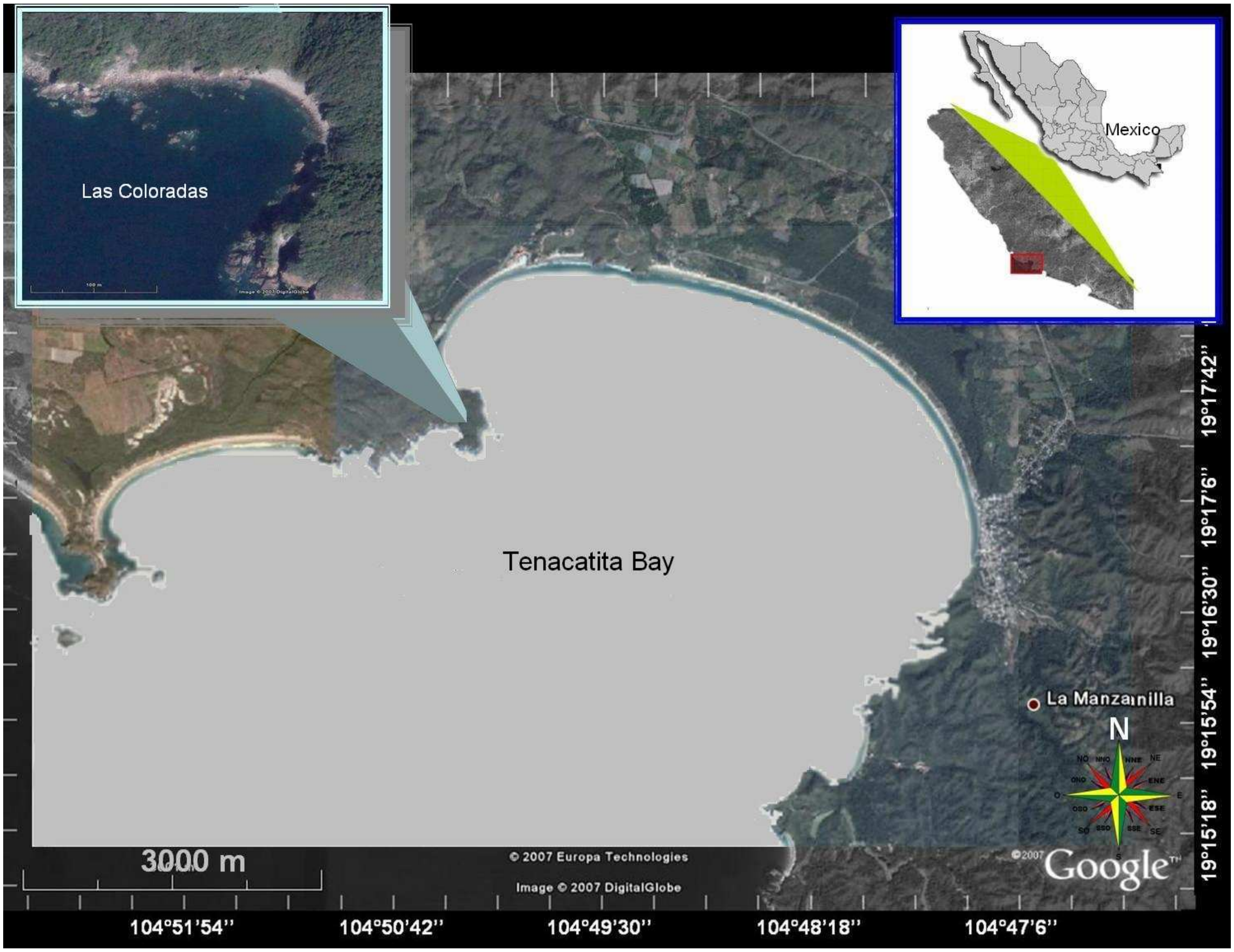

Fig. (1). Study area. Las Coloradas Beach. Tenacatita Bay, Jalisco, México.

transect of 125 linear meters. On each point the geo referenced position was taken through a Global Position System GPS trademark Garmin etrex. Within each segment, of approximately 2 meters wide along the transect, a direct and intensive research of the snails was done. The transect was made parallel to the linear coast, taking into consideration the middle tide level, because the purple snails are preferably distributed at sea level [11, 14].

All the collected snails were taken off the rock manually with all the care needed. They were then counted and biometric data was taken out of each snail, such as: total length (in millimeters, considered from the apex to the most distal point of the anterior siphon canal) done with a vernier digital calibrator; their humid total weight in grams, done with a semi analytic digital balance, and because the purple snail has no sexual dimorphism, sex was determined directly observing the presence of penis or genital orifice, following the method used by Michel-Morfín [19].

Before releasing them, the snails were marked at the top of their shell using aerosol paint color. Once the color dried, all the snails were taken back to the same rocky and coast level zone from where they had been collected.

When they were recaptured, the same procedure was done, but this time using a different color for the snails found within the transect that did not have the previous mark.

The samples were taken during the new moon period in the months of January, February and March 2002. It was done during this time of the year because during these months the sea is calmed and the sea level is low, which assures that the sample conditions are appropriate for the investigation within the intertidal zone. The samples were taken during the hours of the day in which the tide was in its lowest level, this data was verified on the moon tide prediction tables.

We obtained a histogram of frequency of the size distribution for each sample and sex. In order to verify the hypothesis of equality in the distributions, one way analysis of variance and multiple comparison test was done ($p<0.05$). To prove the equality hypothesis on sex ratio an x^2 test was done ($p<0.05$). By using the *t* student test, we did a slope comparison test between the values of the relationship size-weight [20]. The relative density through the linear transect method was calculated by dividing the number of the collected snails between the total considered area [19]. The calculation

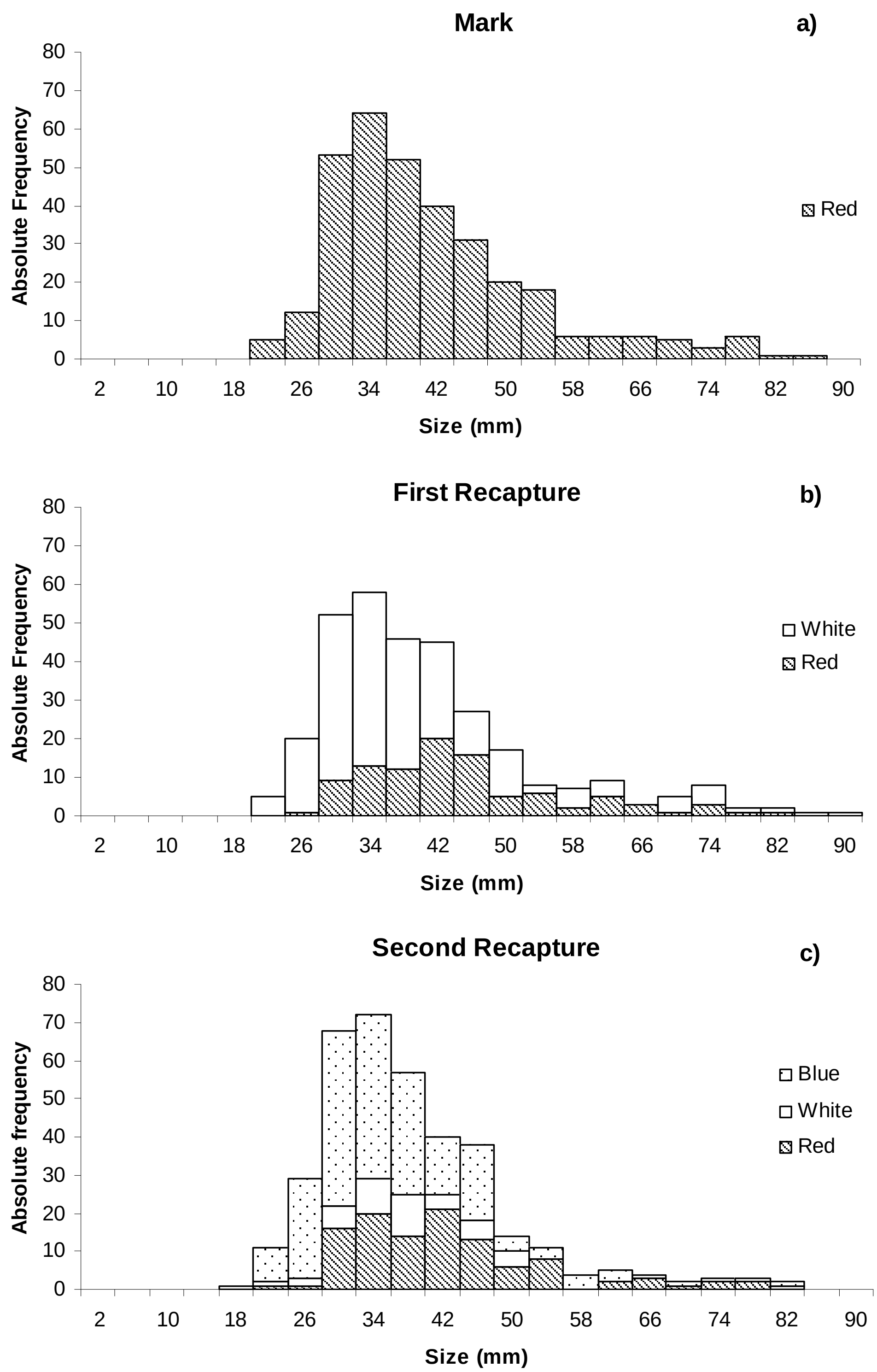

Fig. (2). Lenght frequency histogram of purple snail at (**a**) Mark, (**b**) first recapture and (**c**) second recapture.

to estimate the population size through the mark and recapture method was based on the Petersen, Schnable y Schumacher-Eschmeyer methods [21-23].

RESULTS

In Fig. (**2**), we present the obtained histogram of the frequency of size distribution for each of the taken samples (period). Fig. (**2a**) includes the 329 snails collected in January, all marked with red. The average size is 41.16 mm, with a standard deviation of 12.02 and a range of size between 21.2 and 85 mm. In Fig. (**2b**) we present the snails collected during the second sample, which took place in the month of February, this one is considered as the second recapture. A similar distribution is observed, considering the size of 316 snails with an average of 40.97 mm, a standard deviation of 12.72 and a range between 20.2 and 89 mm. This distribution considers the snails with red mark and those with white mark that was put on them before being released again.

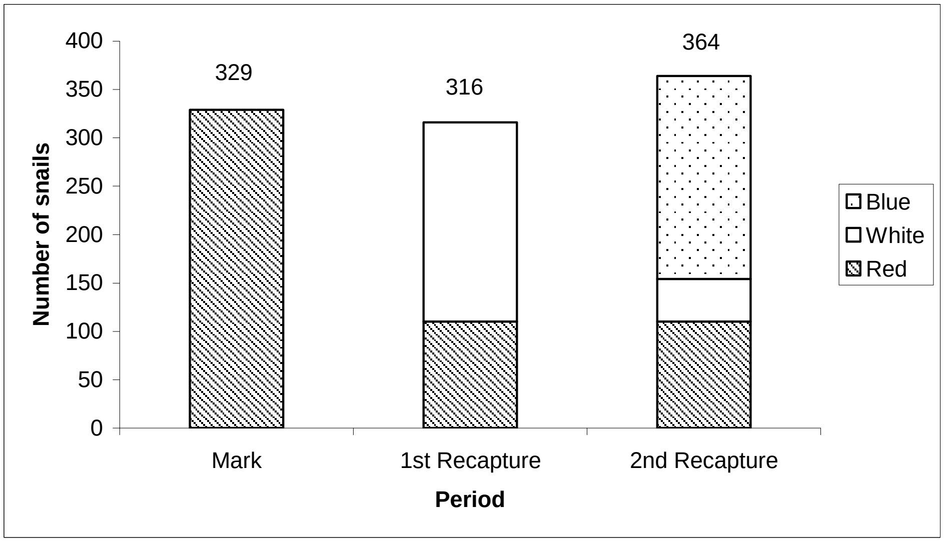

Fig. (3). Total number of purple snail at Mark, first recapture and second recapture.

The size distribution on the second recapture is presented on Fig. (**2c**). In this one, we appreciate a similar tendency of frequency like the previous one. It takes into consideration the size of 364 snails collected during the month of March, the collected snails were marked red, white or had no mark at all. During this time the snails were marked in blue. The average size was 38.41, with a standard deviation of 10.94 and a size range between 18.7 and 83.5 mm.

We can observe graphically that the distribution is apparently similar among the three taken samples. When a variation analysis was done to compare the frequency distribution, significant differences between the distributions have been observed, because at least one average sample is different to the others (α 0.05, P>0.0032).

In Fig. (**3**) we present the total amount of snails per sample. In general the total amount is similar, but it is evident that the snails that were part of the first sample and second recapture are different to the ones initially collected. We also notice that during the second recapture the highest number of collected snails belongs to the group without a mark (210 out of 364). We were expecting a decrease in the number of snails without a mark in each recapture, but this was not the case, therefore it indicates that the population was larger than 336, which is the average number of collected snails from the three samples.

If a detailed analysis of the population structure is done, it is clear and statistically significant the differences observed in the size structure of the collected snails for each sex at each time (Fig. **4**). In general, males have smaller size and disperse less than females.

In contrast to this, Fig. (**5**) shows the relationship between length and weight for each of the samples. Different to the previous graphics, we can observe a similarity in the parameters of the relationship between size and weight between the three samples in the following way; weight = $a*Size^b$. The value of *a* was similar in the three samples (0.0002) and *b* value was near to 2.90, as a good adjustment of the regression coefficient.

The detached information of the composition of each sample, size and sex aspects are presented in Table **1**. We can observe that even if the estimated relative density is similar in the three samples, it is evident that the snails were different on each sample. This can also be reflected in significant changes in sex ratios between each on the samples. During each sample the sex was determined on 111 snails out of 329 collected ones, from this ones 59 were females (53.2%) and 52 were males (46.8%). In contrast to this data, during the first recapture the amount changes dramatically, because from 101 snails, only 34.7% were females and the rest, 65.3% were males. This same tendency was observed during the second recapture, where 36.6% were females and 63.4% were males (*n*=94).

On Table **2** we present the estimated total amount of snails and the relative purple snail's population density through the counting method in transects and through different methods based on capture and recapture data. In these results, it is possible to appreciate that the estimated total number of snails is considerably less through the transect method. This is evident since during the three samples a total amount of 745 snails were different. While the samples were taken 329 organisms were marked, 206 during the first recapture and 210 during the second one (see Table **1**). Consequently, an adequate estimation of the population size should be the same as or higher than 745. This result reveals that the traditional transect method tends to sub estimate the population size and consequently the relative density estimation.

The capture and recapture method data shows a higher estimation of 745 snails, mentioned on the previous paragraph. Therefore the Petersen's method, considering only the capture and recapture data (January and February 2002), show an estimated number of 945 snails. The other methods that consider multiple recaptures (January, February and March); indicate an estimated population higher than 1100 snails in 125 linear coast meters.

DISCUSSION

The mark and recapture method has been frequently used to estimate the growth of different kinds of mollusks [24, 25] and in particular for the purple snail [26]. The method was

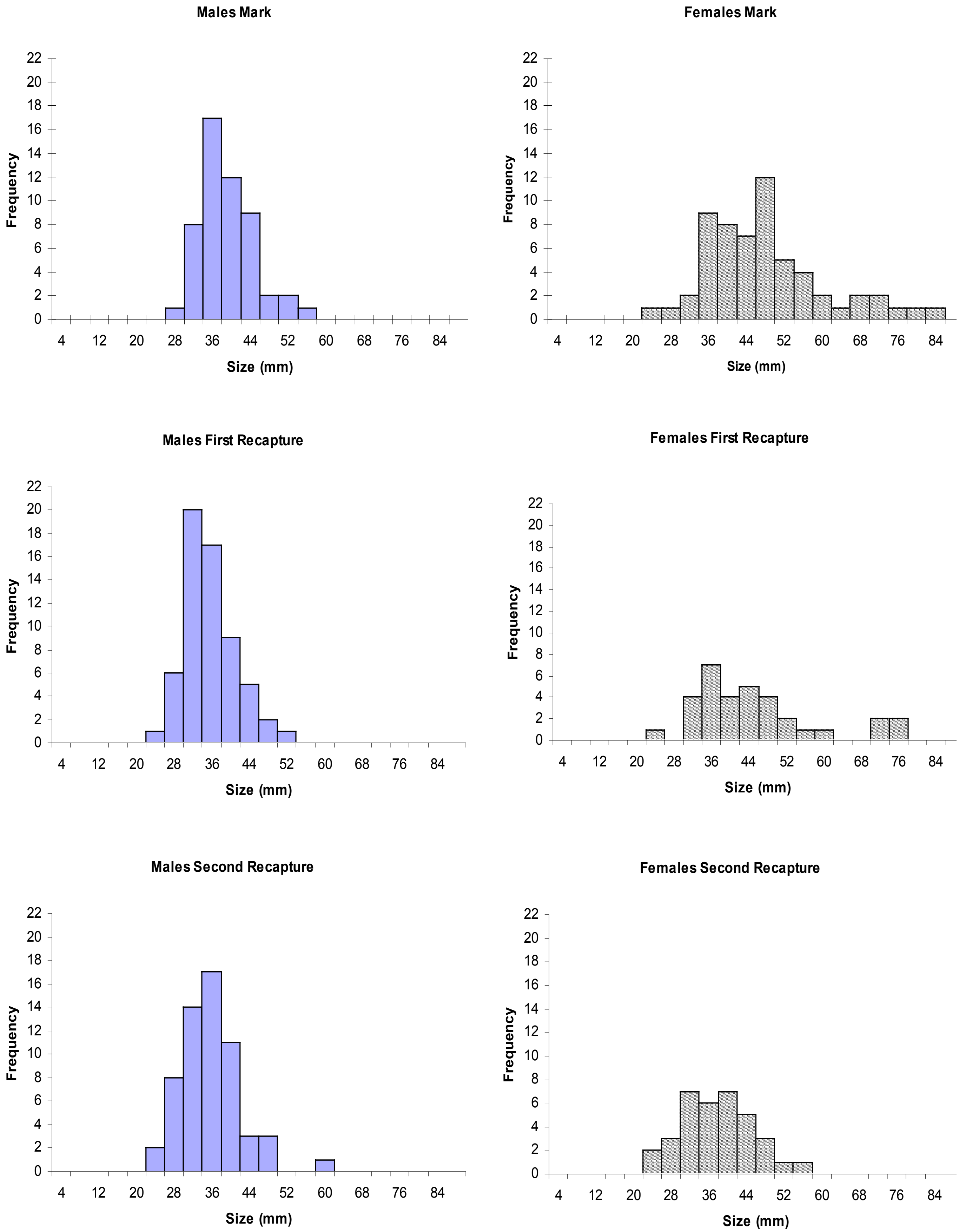

Fig. (4). Lenght frequency histogram of male and female purple snail collected on each period.

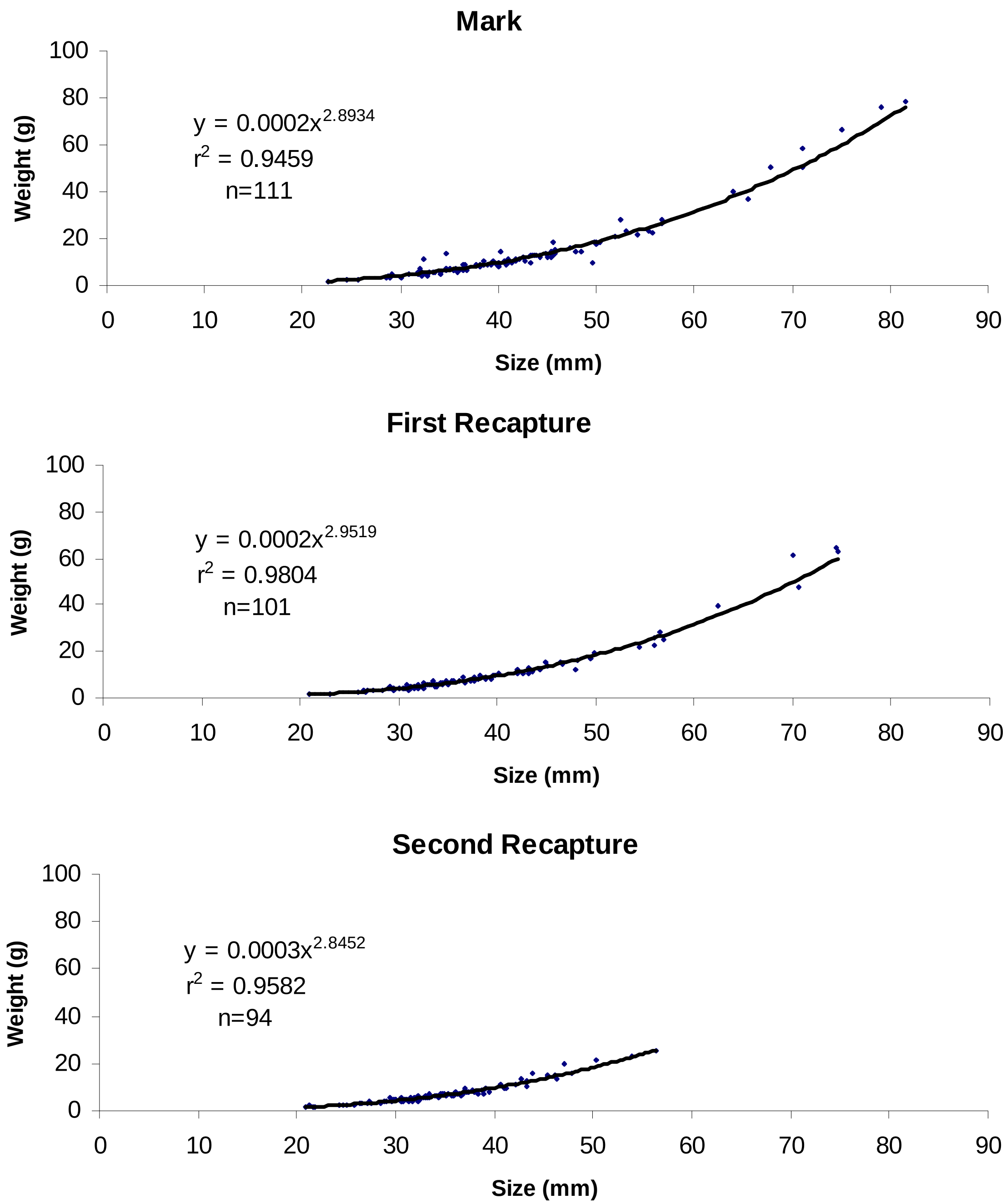

Fig. (5). Lenght- weigth relation for purple snail *Plicopurpura pansa* collected on each period.

used during this test to estimate the population structure and abundance of the purple dye snail *P. pansa*, finding out that the method can be highly appreciated to estimate the abundance in a particular difficult zone to be studied. An intertidal rocky shore, where the habitat is complex having a lot of changes in the sea level and the waves and rocky beach, can contribute to sub estimate the population size, when simple transect methodology is used, a great number of snails can stay out of reach.

Assumptions of Capture - Recapture Method

An important aspect to discuss in this study is about assumptions of capture - recapture method and if these are fulfilled in the purple snail case with a close population, the remaining marks. and the possibility of inducting mortality.

When the test was proposed the first aspect to be considered was if a closed population could be defined, meaning that it could only be affected by the recruitment of new individuals or natural mortality and consequently by free immigration and emigration. In regards to this and although there were no studies that could determine the speed and distance of the snails movement, it is considered that their traveled distance could only reach a few meters. This information was used on observations made within the field and with organisms kept at lab aquariums. To support the idea of considering a close population, a place with a natural delim-

Table 1. Summary of Results Obtained by Mark and Recapture Method to Estimate Population Parameters of the Purple Dye Snail *Plicopurpura pansa* on Tenacatita Bay, Mexico

	Red	Mark Color		Total of Snails	Relative Density (ind/m²)	Average Size (mm)	Stand. Dev. of Size	Sexual Rate (%)	
		White	Blue					Females	Males
Mark	329	-	-	329	1.32	41.16	12.02	53.2	46.8
1st Recapture	110	206	-	316	1.26	40.97	12.72	34.7	65.3
2nd Recapture	110	44	210	364	1.46	38.41	10.94	36.6	63.4

Table 2. Estimation of the Total Number of Snails and Relative Density by Linear Transect and Mark and Recapture Methods

Method	Time	Estimation of the Total Number of Purple Snails	Relative Density ind/m^2
Linear transects.	January 2002	329	1.32
	February 2002	316	1.26
	March 2002	364	1.46
Petersen Method Estimation (Mark-Recapture)	January-February 2002	945	3.78
Schnabel Method Estimation (Triple capture)	January, February, March, 2002	1131	4.52
Schumacher-Eschmeyer Method (Triple Capture)	January, February, March, 2002	1167	4.66

ited area with a rocky beach was chosen to collect the samples. This place was not propitious for the snail presence and acts as a natural barrier.

Beginning with this supposed fact that we are dealing with a close population, another important aspect that has to be taken into consideration is the mark put on the snail's shell. The first question we had was if it would last at least during the recapture periods. Another question was if it would not damage the organisms, and if it would make them vulnerable to plunderers. The chosen option was to apply aerosol paint directly on the snail's shell, once the painting dried out the snails were taken back to the same zone where they had been collected from, leaving them inside the rocks cavity to protect them from the waves and being careful to put them in a humid zone. This assured that the snail handling would not be associated with mortality.

These types of marks have been used in other situations with aquarium snails, observing that the mark stays during several months and does not cause any visible harm. Although it is possible to mark each snail individually putting a plastic number on the labium [19], at this time we decided just to put the mark over the shell. Since we sought to comply with the closed population requirements assumptions necessary for the capture and recapture method, in order to be able to see the number it is necessary to take each snail off the rock. During this study, it was suggested not to take the snails that were feeding themselves or having mattings while the samples were taken, the snails found like this were only counted. The type of mark used is good for short period studies, the possibility of experimenting new mark methods is still open, which could include electronic techniques that could offer a larger number of trusted data in a longer period of time.

A difficult aspect to evaluate is the possible increase of natural mortality rates caused by the mark. It is assumed that the purple snails are captured only by mammals as badgers or raccoons that randomly visit the intertidal zone in search of food and a low probability of increasing the snail's mortality is considered by applying the color on their shell. It is not known of any study that approaches this aspect, but it is assumed that even the natural plundering by these small mammals is not frequent, since no clear evidence, as broken shells, foot prints or teeth can be found in the zone to indicate this.

It was decided to take the snail's sample with a four weeks period between each one, since it has been observed with experiments done within the field and in labs, that mortality can be induced due to the snails handling when they are taken off the rock thus they expel their ink in a period of time lower to 21 and 28 days [5, 27]. One of the most important things of the capture and recapture method is not inducting mortality through the mark or the collecting process, since we wanted to avoid this by all means, therefore we left a four weeks period between the captures.

Comparison of Method

Table **3** presents in a comparative way the used sample method to determine the snail's relative density in different states along the Mexican Pacific coasts. Although other methods have been used such as the quadrant of different areas or the accumulative area method, the one most commonly used has been counting along linear transects, changing the transect length and band wide considered to take the sample.

These differences in the applied methods make it difficult to compare the obtained results and making global inferences

Table 3. Sample Methods and Relative Density Recorded for Several Authors to Purple Dye Snail from Mexican Pacific Coast

Mexican Pacific State	Sample Method	Relative Density (org/m²)	Sampling Year	Reference
Oaxaca	Transect	0.74	1984-1985	A
Michoacán	Quadrant	0.88	1987-1988	B
Jalisco	Quadrant	<2.7	1987-1989	C
Nayarit	Transect 50 x 3 m	2.27	1990	D
Oaxaca	Transect- Quadrant	<2.0	1983	E
Sinaloa	No especified	1.14	1992	F
Isla Socorro	Variable distance transect	0.18	1992	G
Jalisco	Transect 50 x 2 m	1.65	1991	H
Bahía de Banderas, Jalisco	Acumulative areas	1.14	1997	I
Baja California Sur, Jalisco, Oaxaca, Isla Socorro	Transect 50 x 2 m	1.7	1997-1998	J
Guerrero	Transect- Quadrant 30 x 2 m	4.7	2000	K
Guerrero	Transect- Quadrant 30 x 2 m	5.19	2000-2001	L

A [1], B [13], C [14], D [7], E [15], F [16], G [17], H [7], I [18], J [10], K [11], L [12].

about the purple snail's population. Therefore, it is important to evaluate the most commonly used methods and discuss the possible causes of the differences, as well as the importance of these discrepancies in order to assess the species.

The obtained results to estimate the abundance of the population, confirms the hypothesis that the linear transect method sub estimates the real population size and important differences, which are produced in estimating the population parameters. There are certain considerations that have to be taken into account in order to determine the population size of mobile invertebrate species which habitat in an intertidal rocky shore.

First of all, the vertical zone of the benthonic organisms exists, presenting distribution bands of the principal species [28-30]. The length of each band will be determined by the rocky zone slope and by the tide wideness in each particular zone. Therefore, sometimes by considering two meters transect wide, will mean considering the whole snail's distribution zone, while other times it will only be a small part of the zone.

This, together with the tide level at the moment, the population estimation will be determined not only by the inherent factors of the tide for a certain zone, but also by the variations caused by the local wind or storm waves. These factors generally increase the tide level, which means that the snail zone will be partially or totally under water at the moment of collecting the snails.

In contrast, during the lowest sea tide period, the zone can be exposed by the increasing level of insolation or even to an abrupt decrease of salinity because of rain and fresh water flood coming from tropical dry forest around the coast. Regarding these changes it has been observed in several occasions during the field work that the *P. pansa* snail can react by moving itself vertically through the zone, until finding a place that will cover its ecological requirement, generally near to a humid zone.

The vertical distribution of the snail in a rocky zone is an aspect that hasn't been approached by many investigators. León Álvarez [14] found out at Cuastecomates, Jalisco, a rocky zone bay, that the big size snails, thus females, have a preference for the middle tidal zone. García-Ibañez *et al.* [11] approaches this aspect in several coasts of Guerrero, without making sex discrimination and finding out that the organisms of bigger length and weight keep a preference for the middle tidal fringe.

Some environmental aspects such as drying out and wave exposure can influence the gastropods intertidal distribution and the morphological differences of the individuals tend to increase as these gradients increase. The changes occurred in the individuals of the same species related to this gradient can be interpret as phenotypic expressions caused by the environmental stress and sometimes they reveal through the development of a bigger size and the area surface or sticking ability to the substrate [31-33]. This is, bigger sizes at low sea level and exposed beaches, medium sizes at intermediate levels and small sizes at higher levels or zones where the waves splash.

It is also known that the snails do not distribute themselves randomly within the sea zone, but they present an aggregate distribution [1, 14]. This behavior can be noticed at their reproduction time, because during this period we can observe female snails surrounded by male snails, which can be distinguished for their smaller size.

For the relative density estimation done through the transect method, it is not possible to determine the type of the species distribution, through calculating the exponents used in Marine Ecology studies, such as the variance / mean ratio or the Morisita index [34]. The capture, marking and recapture method used in this study, does not allow us to know the population dynamic aspects of these species. In order to do this it would be necessary to use a transect quadrant method. Each method begins with different believes and the outcome will be different, because they will be according to the given hypothesis of the study.

The vertical gradients of the physical and biological factors have a strong influence over the benthic intertidal organism distribution, either sessile or mobile. The density, size and distribution of the different species that coexist in such zone as well as the control, is related to their capability of adjustments and their ability to adapt to such factors [31, 33, 35].

On the other hand, a problem that has been detected in the purple snail population is that the people living in the coastal communities near the rocky zones, travel frequently around this areas to collect chiton (*Chiton articulatus* Sowerby, 1832), Chinese snail (*Hexaplex (T) princeps* (Broderip, 1833)), cap snail (*Calyptraea (T) spirata* (Forbes, 1852)), conch, limpet or other kind of invertebrate as the *P. pansa* bigger than 40 mm. They use them to prepare commonly known "snail's cocktails". This has an impact over the *P. pansa*, since it is a carnivorous that feeds itself from other invertebrates and this anthropogenic activity affects its feeding availability. It even has an effect over the population structure, as it can be observed in the frequency of sex distribution histogram (Fig. **5**), the ones that are over 50 mm are mainly females. Nevertheless, a place with low human activity was chosen, so it is considered that this did not have any effects during the experiment period.

Once the study finished, the limitations of the linear transect method used to determine the relative density and the population parameters of the purple snail's are clear. Due to the fishing, cultural and historic importance of this resource, it is very important to obtain reliable and periodic appreciation of the population size in order to determine the natural or anthropogenic changes of the population. Therefore, a Mark recapture method should be used, as well as a specific designed method in order to get to better knowledge of the species dynamics.

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