



Morphophysiological Indices of the Green Abalone *Haliotis fulgens* Philippi, 1845 at Mexican Ocean Pacific Coast

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Abstract

The monitoring on abalone banks has been intensified since the declining of wild global production due to overfishing, disease, environmental changes and poaching. This is to support timely decisions to execute actions for mitigation, prevention, conservation and fisheries production benefits. Eight morphophysiological indices for green abalone, *Haliotis fulgens* Philippi, 1845 from the Pacific coast of Mexico were evaluated and their performance were elucidated due to their potential as monitoring tools. The Boolootian's gonadic index (GI_B) and gonadic area index (GAI_A) were informative of complete reproductive cycle, thus are reliable for reproductive patterns monitoring. While gonadic index (GI) and gonadic index of calculated mass (MGI) were suitable indicators only of spawning. The condition index of calculated mass (MCI), foot index of calculated mass (MFI), condition index (CI) and foot index (FI) proved temporal changes of soft tissues condition. The MFI clearly defined the best nutritional status of the green abalone foot between March to August, indicating the time when fishing organizations can get the best yields and highest foot quality at Baja California Peninsula, Mexico. Therefore MFI can be used as cheap and quick information to make decisions on the start-up of fishing operations and abalone condition monitoring.

Keywords: reproductive indices, condition indices, foot index, Gastropoda, abalone fishery.

Introduction

Abalone is considered a luxury seafood with high economic value in a complex global abalone market. There are about 75 species of abalone worldwide and less than one third are of commercial importance (Gordon and Cook, 2013). Since 70's, the global wild fishery production has been declining and nowadays represents a relatively small proportion of the world supply which is dominated by aquaculture (Gordon and Cook, 2013). Mexico was the tenth producer of wild abalone in the world in 2010 (Gordon and Cook, 2013), and its production rely primarily on *Haliotis fulgens* (green abalone) and *H. corrugata* (pink abalone) since 1940 (Guzmán-del Proo, 1992; Sierra-Rodríguez *et al.*, 2006). Mexican abalone fishery regulations are catch bans, minimum legal sizes and catch quotas (Guzmán del Proo, 1992) defined annually according on abundance of abalone banks by National Fisheries Institute (INAPESCA, for its acronym in Spanish). In the global context of the

decline of abalone wild production due to a combination of overfishing, disease, environmental changes and poaching (Gordon and Cook, 2013), the morphophysiological monitoring on abalone banks has been intensified, to get additional information on the productivity of populations and insights into the physiological vulnerability of individuals for diseases or dead, as skinny and weak abalone. This information can be used to support timely decisions to execute actions for mitigation, prevention, conservation and fisheries production benefits.

The morphophysiological data gathering is desirable to be cheap and quick, due the abalone fishing regions are extensive and the time to make decision is short, only in Mexico are more than 1 000 km of litoral line. In this sense, morphophysiological indices (MI) could be relevant and low-cost indicators about the nutritional condition or the status of physiological processes (Crosby and Gale, 1990), after overcoming biases of estimating MI from biometric data such as body measurements and organ

weights, due to aspects such as thickening by shell regeneration, or epibionts on shell (algae, oysters, barnacles, drilling organism, etc.), and/or the difficulties to extract the soft parts because of the shell structure, and to isolate individual organs, such as separating the gonad from the hepatic gland.

In this regard, it is important to review, test and define the available options for estimating MI. In haliotids, these indices have been used primarily to evaluate the reproductive status of populations (Booolootian *et al.*, 1962; Webber and Giese, 1969; Poore, 1973; Ault, 1985; Capinpin *et al.*, 1998; Counihan *et al.*, 2001; Litaay and De Silva, 2002; Najmudeen and Victor, 2004; Setyono, 2006). The simplest indicator is calculated by dividing the gonad weight by the total weight of the body, and it is commonly used in haliotids (Setyono, 2006) and other gastropods (Zetina *et al.*, 2000; Morais *et al.*, 2003; Abidli *et al.*, 2012). The indices most commonly used are those that evaluate gonadal maturity based on the cross-sectional area occupied by the hepatic gland-gonad complex (HGGC) in relation to either total shell length (Booolootian *et al.*, 1962; Gurney and Mundy, 2004) or total area (Newman, 1967). Other indices evaluate gonadal volume in the HGGC relative to the specimen's weight (Ault, 1985; Leonart, 1992).

There is no consensus on how to get best standardize estimates by using some particular index or type of indices. Although in some cases indices are not very accurate, they could be advantageous in terms of less cost and quick estimation, and they could have potential for use in certain purposes. This study's aim was to evaluate the performance of morphophysiological indices calculated from data usually recorded in green abalone fishery surveys, in order to elucidate if some indices explain appropriately the reproductive or nutritional status of exploited green abalone banks.

Materials and Methods

Sources of information.—Monthly samplings were made from September 2011 through August 2013 on green abalone banks inhabiting between 8 and 12 m in deep, from La Bocana coast, Baja California Peninsula (26° 48'11.38"N, 113° 44'09.42"W; commercial catch places). 481 abalones between 67- 200 mm in shell size were dissected for tissue sampling and biometric register of: total shell length, in mm (SLt); total weight, in g (Wt); weight of soft parts (Wsp); weight of the hepatic gland-gonad complex (HGGW); and foot weight (Wf).

The area of gonad and hepatic gland were measure on digital images, using SigmaScan Pro version 5. The images were digitalized from cross-sectional sections (5 µm) of HGGW, obtained by the histological procedure of 10% formalin-fixed, dehydrated, paraffin-embedded and hematoxylin-eosin stained (Humason, 1979).

The maximum soft-part weight (Wmc) was calculated in order to eliminate the noise in index estimates due to seasonally variation of both total weight and soft-part weight of abalone, affecting significantly the turgor of soft parts (pers. obs.). Wmc was calculated based on the potential relationship ($y = 33.8x^{0.297}$) between the maximum Wsp observed (y) and each SLt recorded (x). Wsp is the maximum size that soft parts can reach for a given SLt, assuming shell size is a limiting factor in the short term (seasonal timescale).

Indices.—The indices estimated were selected according to available information (Table 1). The gonadic index was estimated as defined by Abidli *et al.* (2012), but using tissue fresh weight instead of dry weight (GI, Table 1). The gonadic index of calculated mass was estimated to express the proportion of HGGW with respect to Wmc (MGI, Table 1). Also, two gonadal indices were calculated using area measures from cross-sectional of the HGGW: the gonadic area index by Poore (1973) (GAI_A, Table 1) and the gonadic index by Booolootian *et al.* (1962) (GI_B, Table 1) multiplied by 0.001 to scale it down to a 0 – 1 range.

Also the condition index (CI; Table 1), the condition index of calculated mass (MCI), the foot index (FI, Table 1) and the foot index of calculated mass (MFI, Table 1) were estimated. The CI values were calculated based on the index used for *Melongena corona* by Zetina *et al.* (2000) but using fresh weight (Table 1). The FI was calculated to express the proportion of foot fresh weight (Wf) with respect to Wsp, to test it as an indicator of foot turgor. The MCI and MFI were estimated to evaluate status of total soft parts and foot respectively, with respect to Wmc.

Statistical procedures.—Data were processed by sex (males and females separately). Monthly variations in *P* indices were analyzed by analyses of variance (ANOVA) followed by Duncan's multiple comparison tests, using the arcsin√*P*-transformed data, due to theoretical distribution of *P* values departed significantly from normality (Kolmogorov-Smirnov test, $P < 0.05$) (Zar, 2010). The correlation between the indices (temporal variations) was examined with Spearman's rank correlation coefficient (Spearman, 1904) and its statistical significance was evaluated by Student's *t* test for *n* (number of data) and *n*-2 degrees of freedom (Zar, 2010). The Statistica 8.0 software (StatSoft Inc., USA) was used for all the statistical analyses.

Results

Reproductive indices.—These indicators presented monthly significant changes (ANOVA and Duncan test, $P < 0.05$). The months with the highest values (autumn-winter period) were markedly different from the months with the lowest values (Figure 1 and 2). The seasonal trends are described

Table 1. Reproductive and condition indices calculated for species of the genus *Haliotis* and other gastropods

Species	Index	Author
<i>H. cracherodii</i> ¹ , <i>H. rufescens</i> ^{1,2} , <i>H. fulgens</i> **	Gonad area index ^{1,2,**} : $GI_A = GA/SI$; Hepatic area index ¹ : $GH = HA/SI$	Booolootian et al. 1962 ¹ , Ault 1985 ² , **this study
<i>H. cracherodi</i>	Gonad index: $GI = Wg/W_{sp}$ Condition index: $CI_1 = Wd/Wt \cdot 100$, $CI_2 = Wd/W_{dt} \cdot 100$, $CI_3 = Wd/Ics \cdot 100$;	Webber and Giese 1968 Zetina et al. 2000
<i>Melongena corona</i>	Gonad index: $GI = Wg/Wt \cdot 100$	Litaayand De Silva 2002
<i>H. rubra</i>	Gonad index: $GI = GA/HGGA \cdot 100$; Gonadosomatic index: $GSI = Wg/W \cdot 100$; Hepatosomatic index: $HSI = Wg/W \cdot 100$	Djoko 2006 ¹ , Capinpinet al. 1998 ² , Ault 1985 ³ , Abidli et al. 2012 ¹ , Counihan et al. 2001 ² , Poore 1973 ³ , Ault 1985 ⁴ , **this study
<i>H. asinina</i> ^{1,2} , <i>H. rufescens</i> ³	Gonad index: $GI = EGV/BW$	Moraisa et al. 2003 ¹ , Najmudeen and Victor 2004 ² , Najmudeen 2007 ² , Elhasni et al. 2013 ³ This study
<i>Bolinus brandaris</i> ¹ , <i>H. asinina</i> ² , <i>H. fulgens</i> ** <i>H. australis</i> ³ , <i>H. rufescens</i> ⁴	General condition index ¹ : $K = Wd/W_{dt}$; Gonadosomatic index ¹ : $GSI = HGGW/Sdw$; Gonadic area index ^{**1,2,3,4} : $GAI_A = GA/HGGA$; Capsule gland index ¹ : $CGI = CGW/W_{sp}$	This study
<i>Patella depresa</i> ¹ , <i>H. varia</i> ² , <i>Bolinus brandaris</i> ³	General condition index ³ : $K = Wd/W_{dt}$; Gonadosomatic index ^{1,2} : $SI = Wg/W_{sp}$ Gonadic index: $GI = HGGW/W_{sp}$;	This study
<i>H. fulgens</i>	Condition index: $CI = W_{sp}/Wt$; Foot index: $FI = Wf/W_{sp}$	This study
<i>H. fulgens</i>	Gonadic index of calculated mass: $MGI = HGGW/W_{mc}$; Condition index of calculated mass: $MCI = W_{sp}/W_{mc}$; Foot index of calculated mass: $MFI = Wf/W_{mc}$	This study

Weight of the gonad (Wg), Weight of the soft parts (Wsp), total weight (Wt), Total dry weight (Wdt), Dry weight of soft parts (Wd), Dry weight of the shell (Ws), Internal capacity of the shell (Ics), Total weight (TW), Estimated gonad volume (EGV), Gonad area (GA), Hepatic area (HA), Shell length (SI), Area of the hepatic gland-gonad complex (HGGA), hepatic gland-gonad complex weight (HGGW), Shell dry weight (Sdw), Capsule gland weight (CGW), Body weight (BW), foot weight (Wf), estimated maximum weight of the soft parts (Wmc).

below.

- Autumn-winter period (September to December 2011): GI and MGI showed an up-down trend (suggesting gonadal development and spawning); GI_B and the GAI_A tended to increase (suggesting gonadal development); in both sexes (Figure 1 and 2).

- Autumn-winter period (September 2012 to January 2013): GI and MGI showed a decrease-increase change (suggesting gonadal decrease and development); GI_B and GAI_A presented an up-down variation (suggesting gonadal development and spawning); in females and males (Figure 1 and 2).

- Spring-summer period (February to September 2012): GI and MGI showed stable values (suggesting gonadal rest, in males and females); males's GAI_A and GI_B increase clearly (suggesting gonadal development); females's GAI_A and GI_B maintain its values during February to May and the increase begins from June (suggesting gonadal rest and development) (Figure 1 and 2).

- Spring-summer period (April to August 2013): all indices tended to increase on final months (suggesting gonadal rest and development, in both sexes, Figure 1 and 2).

The GI, MGI and GI_B showed positive trends between them, and Spearman's R correlations among

GI and MGI were statistically significant in females and males (Table 2). The GAI_A displayed negative tendency respect to others indices, having statistical support those correlations with males's GI_B and females's GAI_A and MGI (Table 2). The MGI had a lower standard deviation compared to the other reproductive indices.

Nutritional indices.—Values obtained for nutritional indices revealed monthly changes in specimen condition, and some changes were statistically significant (ANOVA and Duncan test, $P < 0.05$, Figure 3 and 4). Overall, nutritional indices showed highest average values from March to August in males and females (spring-summer period) (Figure 3 and 4). The seasonal trends are described below.

- Females's FI and CI did not show clear temporal trends (Figure 3).

- Autumn-winter period (September to December 2011): CI, MFI and MCI showed a down-up trend (suggesting nutritional depletion and recovery, in males) (Figure 4); males's FI and females's MCI increased and then decreased (suggesting nutritional recovery and depletion) (Figure 3 and 4); females's MFI was stable (Figure 3).

- Autumn-winter period (September 2012 to January 2013): FI and MFI did not present a defined trend in males (Figure 4); males's CI and females's

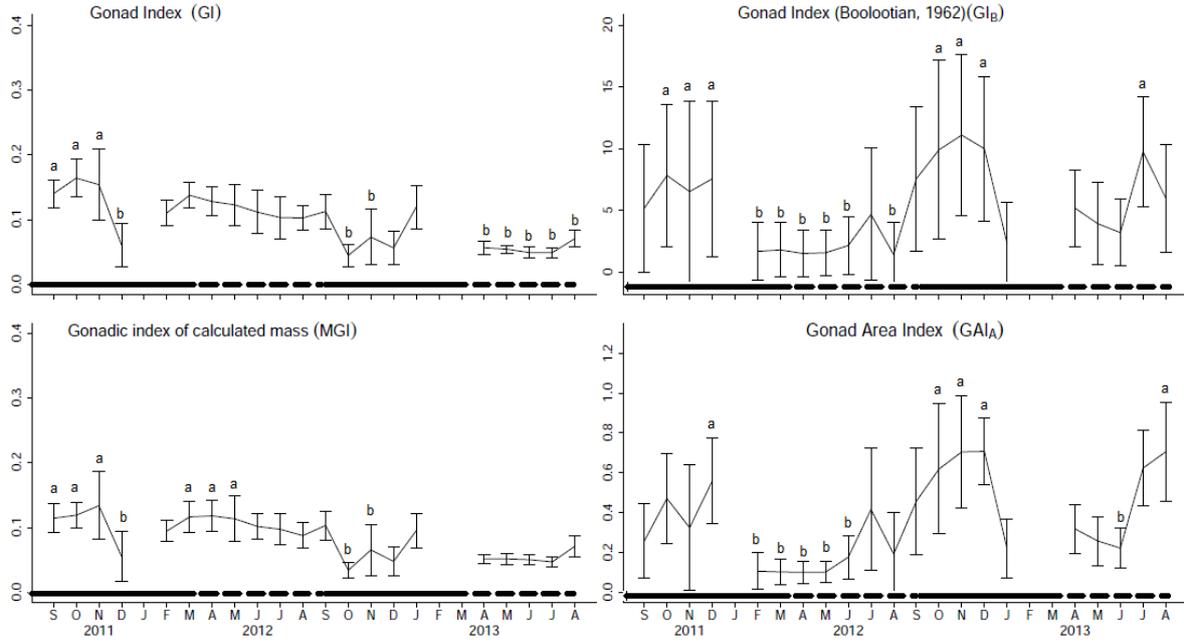


Figure 1. Values of different gonadal indices calculated for female *Haliotis fulgens* - A) Gonad index, B) Gonad area index, C) Gonad Index calculated according to Boolootian, 1962, D) Gonadic index of calculated mass. Graphic symbols: bars (standard deviation), unequal letter on bars (statistical differences, $P < 0.05$), continuous line (autumn-winter period) and dotted line (spring-summer period).

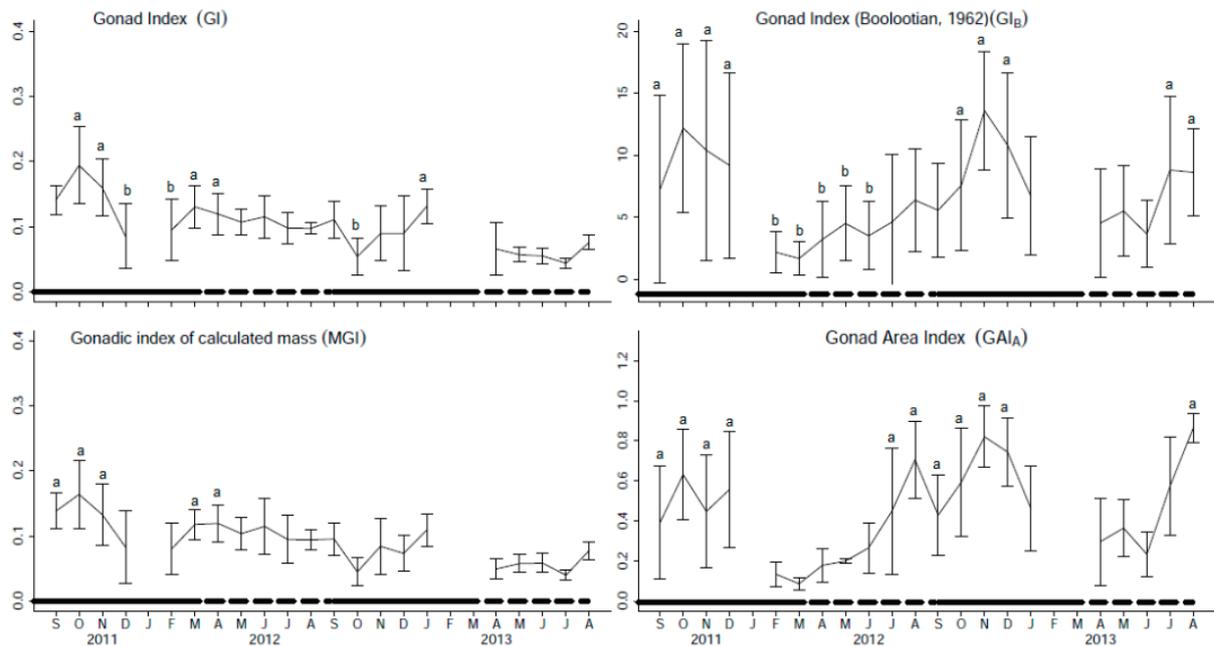


Figure 2. Values of different gonadal indices calculated for male *Haliotis fulgens* - A) Gonad index, B) Gonad area index, C) Gonad Index calculated according to Boolootian, 1962, D) Gonadic index of calculated mass. Graphic symbols: bars (standard deviation), unequal letter on bars (statistical differences, $P < 0.05$), continuous line (autumn-winter period) and dotted line (spring-summer period).

MFI had a down-up change (suggesting nutritional depletion and recovery); MCI showed an increase-decreased variation in males and females (suggesting nutritional recovery and depletion) (Figure 3 and 4).

- Spring-summer period (February to September 2012): MFI and MCI in both sexes and males's FI average values showed an up-down trend (suggesting nutritional recovery and depletion) (Figure 3 and 4);

Table 2. Statistical correlations (Spearman’s rank correlation coefficient, R) between reproductive indices

	Females		Males	
	Spearman R	p-level	Spearman R	p-level
GI- GI _B	-0.3285	0.1458	-0.0519	0.8233
GI - GAI _A	-0.4597	0.0360*	-0.2155	0.3479
GI - MGI	0.9727	0.0000*	0.9688	0.0000*
GI _B - GAI _A	0.9233	0.0000*	0.87013	0.0000*
GI _B - MGI	0.3909	0.0797	-0.06363	0.7840
GAI _A - MGI	-0.5025	0.0202*	-0.2298	0.3161

GI- Gonad index, GI_B- Gonad index (Booolotian, 1962) , GAI_A- Gonad area index, MGI- Gonadic index of calculated mass, * statistically significant correlation

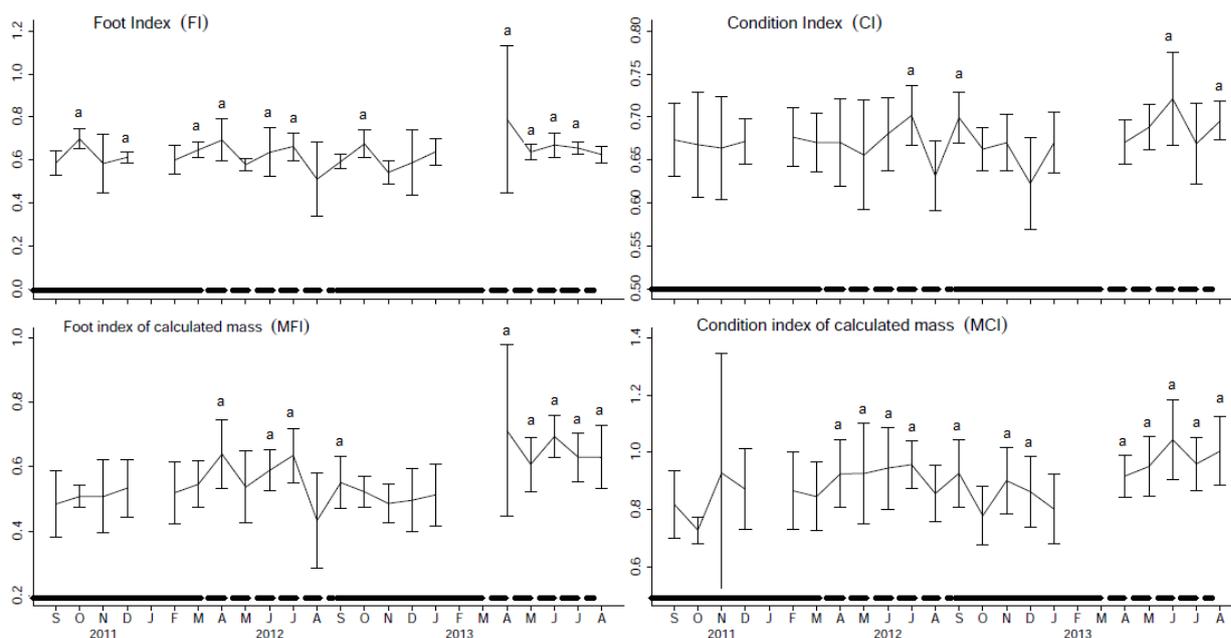


Figure 3. Values of different morphophysiological indices calculated for female *Haliotis fulgens*, A) Foot index, B) Condition Index, C) Foot index of calculated mass, D) Condition index of calculated mass. Graphic symbols: bars (standard deviation), unequal letter on bars (statistical differences, P<0.05), continuous line (autumn-winter period) and dotted line (spring-summer period).

CI did not showed a defined tendency in males (Figure 4).

- Spring-summer period (April to August 2013): all indices presented higher values with respect other months; CI, MFI and MCI of males and females’s MCI values tended to increase on April to June (suggesting nutritional recovery); females’s MFI was stable (Figure 3 and 4).

Most nutritional indices showed positive trends between them in males and females, and only between males’s FI and CI presented a negative correlation value (Table 3). The MCI presented significant statistically correlations with MFI and CI in females and males. The Spearman’s R between FI and MFI had statistical significance in both sexes, and also among MFI and C of females.

Discussion

Reproductive indices.—Reproductive indices provide indirect information that allows to evaluate the reproductive condition, and are commonly used as tools to evaluate gonadal development and determine the timing of reproductive activity (Booolotian *et al.*, 1962; Litaay and De Silva, 2002; Morais *et al.*, 2003; Najmudeen and Victor, 2004; Najmudeen, 2007; Abidli *et al.*, 2012; Elhasni *et al.*, 2013). Usually, a drastic decrease in a reproductive index is interpreted as spawning occurrence (Booolotian *et al.*, 1962). Most indices evaluated in this study consistently showed up-down trends, with notable reductions during or on late autumn-winter period (September to February), suggesting the occurrence of spawning, and coinciding with the spawning temporality documented by Sevilla (1971) at the Isla Cedros, Mexico and Vélez-Arellano *et al.* (2016) at northwest of Baja

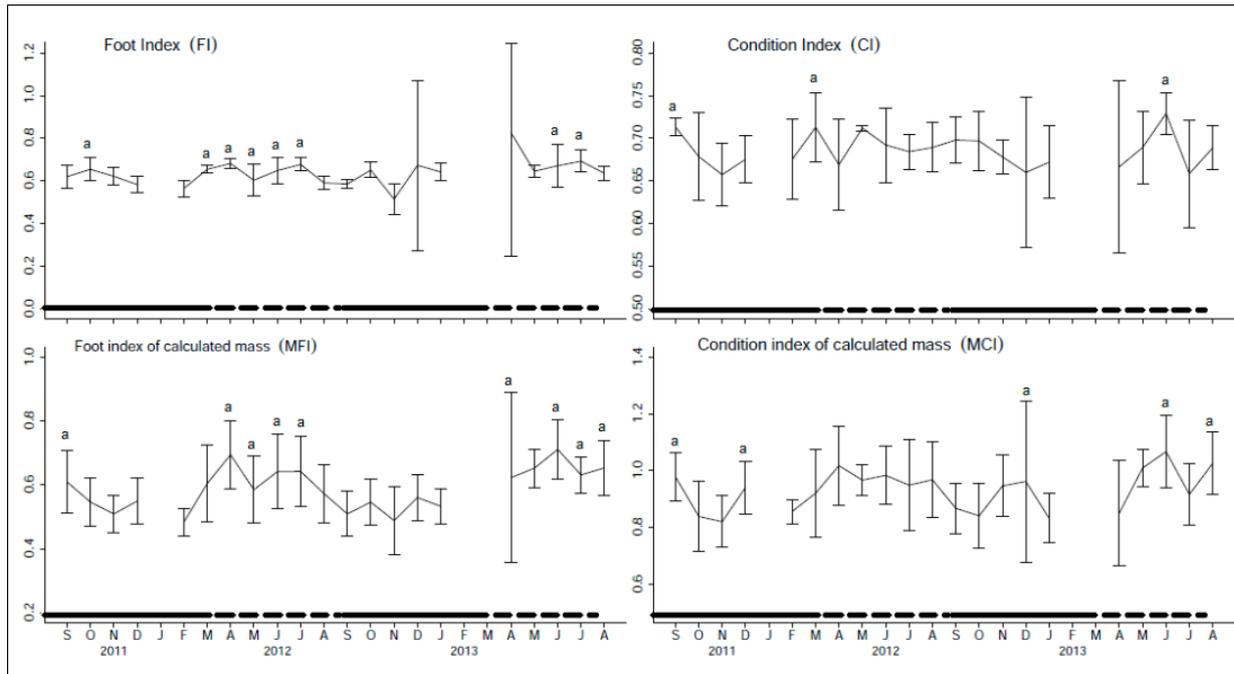


Figure 4. Values of different morphophysiological Indices calculated for male *Haliotis fulgens*, A) Foot index, B) Condition Index, C) Foot index of calculated mass, D) Condition index of calculated mass. Graphic symbols: bars (standard deviation), unequal letter on bars (statistical differences, $P < 0.05$), continuous line (autumn-winter period) and dotted line (spring-summer period).

Table 3. Statistical correlations (Spearman's rank correlation coefficient R) between condition indices

	Females		Males	
	Spearman's R	p-level	Spearman's R	p-level
FI - MFI	0.6519	0.0013*	0.6038	0.003*
FI - CI	0.2506	0.2731	-0.227	0.3218
FI - MCI	0.0298	0.8977	0.0558	0.8099
MFI - CI	0.5831	0.0055*	0.2493	0.2756
MFI - MCI	0.6870	0.0005*	0.7545	0.0000*
CI - MCI	0.5610	0.0081*	0.4337	0.0494*

FI- Foot index, MFI- Foot index of calculated mass, CI- Condition index, MCI- Condition index of calculated mass,

*significant correlation

California Sur, Mexico.

In spring-summer (march-august), indices showed two different trends: maintaining (IG and MGI) and increasing (GAI_A and GI_B). During this period, no spawning activity has been confirmed (Sevilla, 1971; Vélez-Arellano *et al.*, 2016). In this context, the high and stable values of IG and MGI were reflecting the status of the hepatic gland-gonad complex (HGGC), due to a fat hepatic gland instead of gonad maturity. In this sense, if IG and MGI are calculated using the weight of the HGGC, the hepatic gland mask the gonad status outside spawning season. The GAI_A also can mask the gonad development signal (underestimation) due to fattening of hepatic gland (Najmudeen, 2007) or when the gonad area remains unchanged because the trabeculae do not shrink after gametes are released (overestimation) (Ault, 1985). But in this case, the increasing trend of

GAI_A suggests gonads development prior to spawning season in autumn-winter, as well as the GI_B . For its part, GI_B is not affected by changes in the hepatic gland (Gurney and Mundy, 2004) as it uses total shell length as denominator (Booolootian, 1962).

In practical terms, GI and MGI offer only the possibility to detect spawning (through reductions) without having to separate the gonad from HGGC, being reliable reproductive indicators only of spawning. While GAI_A and GI_B suggest the most consistent trends according to the seasonality of the complete reproductive cycle. Also GAI_A is accurate enough to reveal changes related to spawning and partial spawning in *Bolinus brandaris* and *Haliotis rubra* (Abidli *et al.*, 2012; Litaay and De Silva, 2002). And the efficiency of GI_B as an indicator of gonadal maturation also has been demonstrated in *H. cracherodii* and *H. rufescens* (Booolootian, 1962; Ault,

1985).

In addition to GAI_A and GI_B , there are other reproductive indices that are calculated based on variables measured in cross-sections and other biometric data (Ault, 1985; Capinpin, 1998; Litaay and De Silva, 2002; Setyono, 2006; Abidli et al., 2012). This is the case of the gonad bulk index (GBI_{ic}), which is calculated from the estimated gonad volume and body weight (Ault, 1985), and the gonad bulk index ($MGBI_{ic}$), which is computed using area measurements, instead of linear measurements, to estimate the size of the two concentric cones (Leonart, 1992). It is worth mentioning that in a laboratory study, $MGBI_{ic}$ revealed changes in the gonadal development stages of *Haliotis laevigata* earlier than GAI_A , even though both indices were able to identify changes in ovarian development (Leonart, 1992). However, in this study we were unable to estimate and evaluate the performance of $MGBI_{ic}$ and GBI_{ic} , given that measurements of HGGC length were not available, because it is not usually recorded during abalone fishery surveys. These reproductive indices show a high level of accuracy, which might be desirable for studies demanding a more rigorous quantitative approach, including studies under culture conditions. But, on the other hand, their calculation demands more time, as histological procedures or imaging analyses are required for measuring the variables required. For monitoring programs where immediate massive information is required, these indices might be disadvantageous, as their estimation in the field campaigns becomes difficult.

Nutritional indices.—Nutritional indices have been used to assess the nutritional status of fish (Bolger and Connolly, 1989), bivalve molluscs (Crosby and Gale, 1990; Angel-Pérez et al., 2007) and gastropods (Zetina et al., 2000; Abidli et al., 2012), among others. In gastropods, the condition index has been used to evaluate the condition of *Melongena corona* (Zetina et al., 2000) and *Bolinus brandaris* (Abidli et al., 2012) in relation to the reproductive cycle, since it is deemed a good indicator of the maturity state because of the use of muscle and digestive gland reserves in gametogenesis (Webber and Giese, 1969). Angel-Pérez et al. (2007) used a muscle yield index and related it to the gonadal development stages in the bivalve *Atrina maura*. These authors found an inverse relationship, i.e. higher index figures for undifferentiated and post-spawning specimens, and lower figures when the dominant reproductive stages were gametogenesis, maturation and spawning.

Overall, CI, MCI, FI and MFI showed that green abalone males and females achieve their best nutritional condition from March to August. Such period could be coinciding with resting or developing gonads process (gametogenesis), due to no spawning activity has been confirmed (Sevilla, 1971; Vélez-Arellano et al., 2016). Also the abundance of algae (a food source for abalone) at La Bocana increases, as in

nearby areas such as Punta Eugenia (Parada et al., 2009) and Santa Catalina Island (Miller and Engle, 2009). This suggests that the increase in muscle or other reserve tissues is related to a low reproductive activity (maturity and spawning) coupled with high food availability, as energy intake exceeds energy demand (Morais et al., 2003). In this sense, females are likely subject to greater energy loss because of energy-demanding processes such as oocyte production; this is seemingly reflected in the less marked seasonality and the greater variance of indicator values for females relative to males, particularly for MFI and MCI, which showed a more marked seasonality than FI and CI.

Indices calculated using the mass estimated from total shell length (W_{mc}), showed well profiled and seasonally more consistent trends than indices using total weight (W_t) or soft-part weight (W_{sp}) as denominator, because they inherently involve variables which may bias index values, as epibionts or seasonal variations in soft tissues (Avilés et al., 2007; Pedrín-Caballero et al., 2010). Some reports of haliotids shell epibionts are bivalves *Lithodomus* sp. and *Pholadidea* sp. (Crofts, 1929), barnacles (Batham and Tomlinson, 1965), sea sponge (Clavier, 1992) and sabellid worms (Oakes and Fields, 1996). Leonart et al. (2003) reported 35.5 % of weight decreasing due to spionid mud worm infection in *Haliotis rubra* and *H. laevigata*, and Grindley et al. (1998) documented that could occur injures at shells of *H. iris* (16 %) and *H. australis* (38%), like blisters of conchiolin and occasionally necrotic material, forming on the inside of the abalone shell near the apex. Nutritional indices of this study confirm that the soft tissues of abalone exhibit notable changes throughout the year.

In practical terms, MFI and MCI were found as the best nutritional indicators and could be useful for monitoring the nutritional condition and make decisions on the start-up of fishing operations. Because, the abalone mexican fishermen usually try to select the best moment to begin harvest, because they can complete the catch quota capturing fewer abalones and the quality product is culinary better (pers. obs.). The MFI clearly can help to define the best nutritional status of the foot, and for the study period, the index indicated that the feet of best quality were presented between March to August (spring-summer period). This period coincides with the season when fishing organizations have achieved their best yields and the highest foot quality of green abalone at Baja California Peninsula, México.

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